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# Further Remarks on the Intern's Dilemma

*By* Alvin P. Shapiro*

In a recent issue of this journal, an essayist presents the thesis that we are deserting our prerogative to criticize in our urge to be "scientific 'nice guys.' "[1] Whether this situation indeed indicates a major trend towards conformity or merely illustrates that there always have been, and still are, different standards by which people judge success, is a subject either for a statistical study of ways in which one gets ahead in the present era versus the past, or at least for the "good, healthy polemic" which the author suspects is often necessary. But even a polemic, healthy or otherwise, needs an opponent and one of the unfortunate habits of our thinking is the tendency to joust with windmills. The author of another essay in the same journal, concerning psychogenic mechanisms in ulcerative colitis,[2] presents an excellent example of this activity. Criticism is one thing and we may discuss productively whether it is proper or improper, what purposes it serves, and whether we are losing our capacity for "sorting out the relevant from the muck of the plausible."[1] But criticism of opinions which do not exist, or are held only by those who are not aware of the facts, is a betrayal of one's own ignorance of the situation, or at the very least a confession of gullibility.

The author of "Ulcerative Colitis in the Age of the Id"[2] presents the instance of the intern who is puzzled by his inability to elicit evidence of psychiatric disturbance in his patient with obvious ulcerative colitis and accordingly doubts his diagnostic acumen. The essayist goes on to point out that this illustrates a "popular and growing conviction that the cause of ulcerative colitis is a psychiatric disturbance" and draws us a charming picture of an informal diagnostic conference at the country club, where, we presume, a candlelit seance is held to probe the Freudian implications of the case, with much finger-pointing and name-calling.

But whence comes this conviction? Certainly not from the writings of those psychiatric investigators who have most extensively studied and written about ulcerative colitis. Engel, whose exhaustive and brilliant series of articles summarizes the current psychological and somatic data in this disease,[3] repeatedly points out that psychodynamic events cannot be considered etiologic in the usual sense of single causation of disease. His hypothesis goes only so far as to suggest that psychogenic factors may be a necessary component in the development of the illness, in that they may produce physiologic changes in the bowel—vascular, immunologic, or otherwise—which, in the presence of other etiologic mechanisms, culminate in appearance of ulceration. Such a hypothesis allows ample room for the patient in whom the psychologic disorder exists and who is unaffected by colitis, as well as for the well adjusted person—or indeed even the "happy mongolian idiot"[2]—who is a victim. Even the writers whose hypotheses are more classically couched in psychoanalytic terms have on the whole been exceedingly careful to point out that a combination of factors is most likely responsible. That their descriptions include vague terms such as "stock-bound colon reactors," "organ selection," and "vegetative neurosis," is best explained by our ignorance of more specific physiologic mechanisms rather than by a desire for obfuscation. Engel sums up the problem nicely when he states, "these are psychologic processes which may be expected to follow upon the traumatic separation, but whether they have physiologic aspects which specifically contribute to the colitis cannot be established by psychologic means alone. But we must avoid the error of assuming that the discovery of evidence favoring one mechanism necessarily means that other mechanisms are not also operative and perhaps

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contributing to the full development of the pathologic process."[3] Had our enterprising intern read this carefully, would he have been so befuddled?

There are, of course, extremists who try to explain somatic phenomena in terms which excessively "psychologize about physiology and biochemistry."[4] But among the productive groups in psychosomatic investigation, these men hopefully are the exceptions, and the fallacies of their work are recognized with no greater delay than in other disciplines. The rules of scientific verification are not unknown to the investigators in this field. If the tools and methods of the research appear difficult for the untrained to use or understand, let us not forget that all of us are not able to manipulate a cardiac catheter, nor understand the nuances of the biophysical and mathematical concepts concerned with the study of body fluid distribution with radioactive isotopes. The investigator in psychosomatic disorders is not attempting to establish an "either-or" concept, but rather endeavors to further understanding of disease processes by utilizing as instruments psychoanalytic and other methods, which he manipulates in order to "uncover the psychologic determinants in various physiologic disorders."[4] The relative importance of the mechanisms he proposes in the totality of pathogenesis can only become apparent when all the facts are in. And the psychosomatic investigator is only attempting to contribute a fact, which he has done with rare success in ulcerative colitis. Nor is he misled to believe, as our controversial author implies, that the proof of his thesis must be demonstration of curative psychotherapy. This is often demanded of him by his medical colleagues, which is indeed a rare form of gamesmanship. After all, no one denies the important contribution of the failing heart to congestive failure when digitalis is no longer effective in restoring compensation.

Arguments over single and multiple cause have waged for years and those interested in psychosomatic disorders find themselves in the situation of needing to defend the position that individual susceptibility varies and the "whole man" must be considered. Yet this unfortunate cliché needs no defense when we consider the etiologic role of individual variation in susceptibility to bacterial disease. After all, is the cause of repeated infection in individuals with multiple myeloma the dysgammaglobulinemia or the invading bacteria? Certainly, we can treat either or both components and (hopefully) favorably influence the course of infection or prevent its recurrence. What unfortunately seems often to develop when psychologic factors are invoked as possible etiologic components is an argument, with many emotional overtones, on the subject "Psychiatry, for or against." It should be hoped that we are beyond the need for this type of debate. Indeed, if "ulcerative colitis is . . . an accusation and not a diagnosis,"[2] who is the accuser, and why must he accuse? What this boils down to is that having admitted of the existence of psychiatric factors, we must discuss their significance rationally, with scientific detachment and not with mystical delight or condemnation.

The argument posed in our essayist's contribution is not new. It is met with on the medical wards daily, as Graham has pointed out.[5] It confuses the issue repeatedly in diseases such as peptic ulcer, hypertension, rheumatoid arthritis, etc. No special fear need be reserved that the "psychiatrically oriented" physician is uniquely qualified to be one who treats for one disease while the patient wastes away of another ailment. He will do this only in proportion to how well or poorly trained a physician he is. In my own experience, and in those of others who have spent considerable time in imparting psychiatric information in medical problems, a considerable percentage of patients referred for psychosomatic consultation are those in whom the obvious emotional problem and/or the difficulties in doctor-patient relationships have resulted in neglect or misdirection of the medical workup.

Our confused intern, who experiences diagnostic skepticism because all the facts are not in order, is to be congratulated. If he does not find all the data, he is in the same position he faces when the patient with obvious signs and symptoms of mitral stenosis—the flushed face, the hemoptysis, and the classical thrill and murmur —offers no history of rheumatic fever. At the least, his technic of anamnesis may need further development; on the other hand, the experience may add to his knowledge that the "textbook picture" is an unusual phenomenon. If these are not sufficient stimuli for him to have the "open mind . . . to solve what *is* thus far an unsolved mystery," then certainly the knowledge of whether the cause is known or unknown will not salvage this fellow's medical curiosity.

In summary, may I point out that a fair appraisal of the available facts, and in particular those culled from essentially psychiatric sources, indicates that no militant attempt to establish a purely psychogenic cause for ulcerative colitis has evolved. To state that such an organized opinion

exists, and to warn against its propagation lest we deny our patients the benefits of steroids and/or colectomy, is to create a straw man. And unfortunately progress is not made, nor the purpose of constructive criticism served, by the creation of straw men whom we can then conveniently destroy.

## REFERENCES


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   d) IV. The significance of headaches. Psychosom. Med. *18*:334, 1956.
4. Mirsky, I. A.: The psychosomatic approach to the etiology of clinical disorders. Psychosom. Med. *19*:424, 1957.
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# Should Science Get ALL Our Keen Minds?

*By* William W. Stead*

One hears on every side that the United States must develop its best minds early and get them into scientific work. It is stated that science must have the benefit of the keenest natural intelligence we can produce in order to surpass the Soviets. Our youth are urged to begin serious study early and not to waste time in adolescent frivolity. It seems evident that the Russians have operated under such a program for some time, if one can judge by what they have accomplished recently in applied science and engineering. And yet even before our most recent call to accelerate scientific study, the fields of physics, electronics, aerodynamics, engineering and rocketry were already so attractive that they were taking an increasing proportion of bright young men. Every medical school admissions committee can attest to this.

But the field in which there is the most serious need for brilliant minds has received very little attention. The real need for a "breakthrough" lies in the field of human relations, i.e. social psychology or behavioral science. We have made greater strides in physical sciences than we have in working out how men can live and work together on this globe. Even if we solve the many serious problems of space travel, navigation and survival under unusual conditions, it will be a long time before emigration from the earth to other planets will be feasible on a sufficiently large scale to be significant. Thus we cannot count upon emigration to remove the pressures of over-crowding, starvation and ideologic conflict that confront us. We must look for a solution here on earth if we are to survive long enough to enjoy the space age.

Money is available from the government and numerous private agencies for the support of scientific projects, education and symposia, the purchase of expensive equipment and travel to meetings. In addition to a record Public Health Service appropriation in 1957, for instance, Congress appropriated twice as much money for medical research as the Chief Medical Director of the Veterans Administration had suggested. It was stated that Congress desired a great acceleration of the attack upon "cardiovascular diseases, cancer, neurology and psychiatry, degenerative diseases and problems involved in the ageing process." Industry supports psychologists for motivational research to guide their advertising programs. But there is almost complete lack of support of education, personnel, and basic research in the field of human relations. Certainly there is no "crash program" in this field. Sound progress is best made under conditions of less duress than can be achieved in "crash programs" and "brain-storming." However, when the physical sciences are racing ahead with such programs it would seem dangerous not to accelerate progress in social matters.

Most scientists who read these lines will probably think that this is all just an impractical, ephemeral thesis without meaning because there really is no substance to "social science." They would reason that the field of human relations is so intangible that it cannot be approached as a science. No argument against this view can be offered at this time except to point out that those of us who work in conventional, basic or applied science have not devoted our greatest efforts to social science. Our inability to see a ready approach to the problems is no indication that one could not be found by a sufficient number of intelligent people devoting their entire attention to the matter.

There are many examples of men having seen no approach to difficult problems which then became simple and commonplace after the "breakthrough" had been made. The conversion of energy and mass back and forth, flying, electronic computation and television would have seemed impossible to conventional scientists of 100 years ago. The attainment of peaceful intercultural human relations now seems almost impossible, but we must remember that man has always done the "difficult today and the impossible tomorrow."

Until the advent of mass communications

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and atomic weaponry there was little urgency about making any really serious attack upon social studies, international relations, population pressures and world trade agreements. Men could dabble in these fields at leisure. But the advent of a nation's capability suddenly to annihilate and be annihilated almost simultaneously has injected real urgency into the matter. There can be a break-through to open up this field only if enough really keen minds are applied to the task. We must devise means of attracting young people into a study of these matters.

Conventional science is more attractive to most of us than is social science. One reason for this is that in physical science there are tools with which to work, established avenues of approach and tangible problems which we can attack. We feel a sense of security in this type of work because it is rather well defined and instrumentation is highly developed. On the contrary, the field of human relations is nebulous and there are no well defined problems or tools with which to work. If the mature members of the population feel insecure with this field it is easy to understand that an adolescent would be extremely reluctant to venture into such an uncharted area. Yet it is the youthful mind that is most apt to crack the problem of how the earth's inhabitants can live peacefully on this planet while we develop means by which some can emigrate to other worlds and relieve the tension. These matters are far more important to all of us than are electrolyte shifts, antibiotic action, or even the great problem of the mechanism of cancer.

There have, of course, been many attempts made to find solutions to these problems of intercultural relations. The United Nations was the great hope of our day, but it seems frequently to bog down in minutiae and lose sight of the real goals. There needs to be a coordinated effort to work out practical ways by which men can live together in the world. At the present time one such effort is being made by Dr. Paul Wilcox, a psychiatrist in Traverse City, Michigan. Dr. Wilcox is privately generating interest in the establishment of an International Human Relations Year in 1970, comparable to the International Geophysical Year. But for any such effort to succeed there must be a great deal of interest by our society, supported by official recognition and financial support.

A frontal attack must be made upon the problems of population pressures, international competition, world trade, etc. If such problems were attacked with as much vigor as was devoted to atomic energy and rocketry, there is a chance that solutions could be found. Probably the Communists would realize the urgency of the situation and join us in a positive program to seek solutions.

It is important for us to examine our situation on the earth carefully in order to determine whether we are wise in channeling all brilliant minds into conventional forms of science or whether we should try to reach out in new directions to bring the scientific method to the solution of man's greatest present need: i.e. survival in the presence of facilities the use of which by either great power would destroy much of the world's population. If this is not done quickly, our civilization may well follow the many before it which have collapsed and disappeared.

# NOTICES

## The 1958 National Meeting

In 1955, the Federation attempted for the first time to provide for the presentation of more papers than can be accommodated on the general program by sponsoring small Sunday evening meetings in special fields. A second objective of these meetings was the stimulation of discussion from the floor, its having become evident that comments, criticisms, and general exchange of information lessen at the general meetings in Atlantic City as attendance increases each year. The success of this venture by the Federation was attested to by the many requests for additional meetings and the enthusiastic participation in them in 1956 and in 1957.

After the 1957 meetings, the Council of the American Society for Clinical Investigation offered to cooperate with the Federation in this type of program. It was finally agreed that in 1958 sectional meetings would be expanded in number and scope, that they would be conducted under the *joint sponsorship* of the two societies, and that the papers for presentation would be drawn from the abstracts submitted to both organizations.

The Federation will hold a general scientific session from 9 a.m. to 1 p.m. on Sunday, May 4, 1958. There will be a very brief business meeting (the only items to be covered will be the voting on a constitutional amendment prepared last year and the election of two new councillors). The various reports usually given by the Secretary-Treasurer are published in this issue and will not be read at the meeting.

The afternoon of May 4 will be devoted to sectional meetings beginning at 2:30 p.m. There will be six meetings running concurrently throughout the afternoon. Three of these will continue in the evening from 8:00 to 10:00 p.m. and a seventh section will meet in the evening only.

The officers and Council hope that this first full-scale departure from the traditional form of the program in Atlantic City will result in the most valuable meetings in the history of the American Federation for Clinical Research and the American Society for Clinical Investigation.

*Ivan L. Bennett, Jr.*
*President*

## Application for Membership

1. Young research workers are encouraged to apply for membership. It is unnecessary to await a member's invitation to join the AMERICAN FEDERATION FOR CLINICAL RESEARCH.

2. There is one requirement for regular membership: publication of a meritorious investigation in clinical medicine or allied sciences. This should not be a case report or an abstract.

3. An applicant must ask a member of the Federation who knows him to sign his application.

4. Interested individuals should write to: AMERICAN FEDERATION FOR CLINICAL RESEARCH, V. A. Hospital, Minneapolis 17, Minnesota.

## New Research Tools

The information reported here is obtained from manufacturers. All notices and inquiries should be addressed to **New Research Tools Editor, Samuel N. Turiel & Associates, Inc., 750 N. Michigan Ave., Chicago 11, Ill.** Include name(s) of the manufacturer(s).

- **Adsorbents** for use in all chromatographic instruments are listed and priced, with advice on proper selection. Burrell Corporation.
- **Protection of personnel** engaged in handling radioactive materials is discussed in pamphlet on health physics. Tracerlab, Inc.
- **Pulse-height analyzer and scaler** combined in single chassis, with high voltage supply and automatic push-button computing circuit. It is designed for use with all gamma-sensitive scintillation counters. Nuclear-Chicago.
- **Radioisotope equipment** for medical use is described in a catalog that indicates which instruments are needed for various research and clinical procedures. Tracerlab, Inc.
- **Scintillation counter** modified for use in spectrometric analysis by permitting output pulses of the photomultiplier tube to be fed directly to the linear amplifiers used for spectrometry. Tracerlab, Inc.
- **High-efficiency acoustic stethoscope,** said to make cardiovascular sounds about twice as loud as do conventional instruments. Sanborn Co.
- **Disposable plastic caps** for micro blood collecting tubes. Scientific Products.
- **Radioactive deanol** (2-dimethylaminoethanol), and four new compounds of Nitrogen-15 (60% nitric acid, ammonium nitrate, potassium nitrate, ammonium sulfate) now available. Tracerlab, Inc.
- **Improved source calibrator** designed for quick determination, in microcuries or millicuries, of liquid or solid sources of radioactive isotopes. Tracerlab, Inc.

• **Recording instrument** allowing simultaneous recording of up to 16 processes. It produces immediately visible dry writing on unprepared paper, and recording pointers glide along a common straight edge. Recording channels are equipped with direct voltage amplifiers. Various paper speeds are possible. Brinkmann Instruments, Inc.

• **Piston burette**, either hand-operated or motor-driven, for titrations. Basic principle is that of a piston displacing a liquid in a cylinder. Brinkmann Instruments, Inc.

• **Screw-cap Erlenmeyer flasks**, making cotton-plugs unnecessary, and providing a means for keeping solutions and media air- and vapor-tight. They are made of heat- and chemical-resistant low expansion glass. Owens-Illinois.

## Reports for Business Meeting May 4, 1958 American Federation for Clinical Research

Because of the change in the form of the meeting this year, the business meeting of the Federation will be very brief. In order to conserve as much time as possible the reports which are customarily read by the Secretary-Treasurer are printed herewith for the review of the membership in advance of the meeting.

*Minutes of the Business Meeting*, May 5, 1957, Dr. L. E. Hinkle, Jr., presiding:

1. Secretary-Treasurer reported on the state of the Federation.

2. It was announced that the Council had elected Dr. A. J. Bollet to the Publication Committee for a term of 5 years.

3. Dr. Paul Van Arsdel of Seattle was elected to the Council for a term of 5 years.

4. The following Amendments to the Constitution were proposed:

(The Constitution requires that any amendment be presented to the membership in such a way that it can be studied over a period of a year before it is brought to a final vote. The following amendments were presented to the membership at the 1957 Annual Meeting, published in *Clinical Research Proceedings* in September, 1957, and are reprinted here so that they can be read by each member before final passage. Unless there is objection from the floor they will not be read aloud before being put to a vote.)

(1) It is proposed that Article III, Section II be modified to read:

There shall be a National Council which shall meet at least annually at the time of the National Meeting. New members to fill vacancies shall be elected each year. (Signed: Arnold S. Relman, Albert I. Mendeloff, G. Watson James, III, Lawrence E. Hinkle, Jr. and George E. Schreiner.)

(2) It is proposed that Article III, Section IV be modified to read:

The Council shall be composed of the President, Vice-President, Secretary-Treasurer, the Chairman and Secretary of each geographic Section, and five Councillors-at-large, one of whom is to be elected each year for a period of five years. (Signed: Albert Mendeloff, Ivan Bennett, Robert P. Gilbert, Franklin H. Epstein and David T. Graham.)

(3) It is proposed that Article III, Section V be modified to read:

Councillors-at-large to fill vacancies shall be nominated at the Annual Meeting by a Committee composed of the Section Chairmen. The recommendation of this Committee and any additional nominations which may be made by the membership shall be presented to the Federation as a whole for decision. (Signed: Albert I. Mendeloff, Ivan Bennett, Robert P. Gilbert, Franklin H. Epstein and David T. Graham.)

*Secretary's Report*

1. Activities of the National Office:

| | |
|---|---|
| A. Action on applications received since May 1, 1957: | |
| Elected to membership | 175 |
| Application rejected | 1 |
| B. Dropped from the roster: | |
| Deaths | 5 |
| Resignations | 20 |
| Dropped—Associate more than 5 years | 2 |
| —Non-payment of dues | 25 |
| *Total* | 52 |
| C. Miscellaneous Activity: | |
| Advanced from Associate to regular Member | 12 |
| Advanced to Senior status | 28 |
| Changes of address | 319 |
| Members lost (mail returned) | 10 |

2. Classification of Members

| | |
|---|---|
| Regular | 1722 |
| Senior | 1102 |
| Associate (Discontinued in 1954) | 54 |
| *Total* | 2878 |

Comparative Figures: 1955: 2290; 1956: 2540; 1957: 2760.

3. The Upjohn Company was elected to

Contributing Membership and the Wyeth Corporation to Supporting Membership.

4. Efforts have been increased to attract more advertising to our official journal, *Clinical Research*. The questionnaire which was sent out in June revealed that a gratifyingly large potential and actual buying power resides in our membership. The results of the questionnaire were reported in the January issue of *Clinical Research*. The buying potential of the readers will be brought to the attention of both instrument and pharmaceutical manufacturers by our advertising agent. Such efforts are necessary because the steadily increasing cost of publication could not be met without increasing dues. It will be extremely helpful if members will mention seeing an ad in *Clinical Research* whenever they write for information about a product produced by one of our advertisers.

5. A special meeting of the Council was held in Chicago on October 31, 1957, for the purpose of working out terms of cooperation with the American Society for Clinical Investigation for the National meeting. Our aim was to improve the quality of the meeting and still increase the total number of papers that could be presented.

6. In September, the Secretary-Treasurer became separated by 1500 miles from the National Office in Minneapolis. All routine matters have been handled by Miss Lila Mitchell in Minneapolis. The portion of administration falling more directly under the Secretary-Treasurer has been handled in Gainesville. It was felt that the expense would be too great to move the National Office for the last nine months of the tour of office of the present Secretary-Treasurer. The physical separation has caused little inconvenience or loss of efficiency.

*Treasurer's Report:*

*Balance Sheet*
*December 31, 1957*

ASSETS

| | | |
|---|---|---|
| *Current Asset:* | | |
| Cash in bank– | | |
| Checking account | $ 2,734.14 | |
| Savings account | 4,000.00 | |
| | | $ 6,734.14 |
| *Investments:* | | |
| United States Savings Bonds | | 4,000.00 |
| *Office Equipment*–at Cost | | 1,441.18 |
| *Total Assets* | | $12,175.32 |

LIABILITIES

| | | |
|---|---|---|
| *Current Liability*–Payroll taxes deducted from employees' salaries | | 282.45 |
| *Surplus*–(See below) | | $11,892.87 |
| *Total Liabilities* | | $12,175.32 |

*Statement of Income and Surplus*
*Year Ended December 31, 1957*

| | | |
|---|---|---|
| *Receipts:* | | |
| Membership dues | $17,527.40 | |
| Special memberships | 6,500.00 | |
| Interest on bonds | 105.20 | |
| Profit on *Clinical Research Proceedings* | 104.25 | |
| Other | 459.50 | $24,696.35 |
| *Expenses:* | | |
| Publications–*Clinical Research Proceedings* | $10,521.62 | |
| Stenographic salaries | 6,802.11 | |
| Meetings | 1,799.33 | |
| Postage and stationery | 1,397.73 | |
| Travel expense (council meetings) | 998.17 | |
| Accounting fee | 250.00 | |
| General expense | 302.26 | |
| Telephone and telegraph | 550.73 | |
| Bank charges | 9.18 | |
| Equipment rental | 95.04 | |
| Publication for Western Society for Clinical Research | 200.00 | |
| Social security taxes | 126.32 | |
| Loss on sale of equipment | 172.58 | 24,455.57 |
| *Excess of Receipts over Expenses* | | 240.78 |
| *Surplus*, January 1, 1957 | | 11,652.09 |
| *Surplus*, December 31, 1957 | | $11,892.87 |

Respectively submitted,
*William W. Stead, M.D.*
*Secretary-Treasurer*

## Medical Writing Award

An award of five hundred dollars for "the best unpublished manuscript for a short book on a clinical subject in the field of internal medicine" is offered by *Modern Medical Monographs*, published by Grune & Stratton, Inc. The winning monograph, if found suitable, will be published as a book. The author must be a graduate physician less than 40 years of age. Additional information may be obtained from Dr. Richard H. Orr, 37 East 67th St., New York 21, N. Y.

## Fellowship

The L. N. Upjohn Fellowship in Experimental Therapeutics is being offered again at the University of Oklahoma School of Medicine. It is available to those who have completed resident training in Medicine or Pediatrics, and who plan a career in Clinical Investigation. The stipend is $6000 per annum, beginning July 1, 1958. Address inquires to Dr. Stewart Wolf, 800 Northeast Thirteenth Street, Oklahoma City, Oklahoma.

## Members Lost to the National Office

The current address of the following members is unknown:

| | |
|---|---|
| Louise A. Desy | 84 Commonwealth Ave. Boston 16, Mass. |
| Capt. Hushang Javid, M. C. | Madigan Army Hospital, Tacoma, Wash. |
| Dr. Henry J. Koch, Jr. | Anderson Hospital Houston, Tex. |
| Dr. Carl J. Marienfeld | Dept. of Pediatrics U. of Illinois 1819 W. Polk St. Chicago, Ill. |
| Dr. Carroll E. Roach | Eli Lilly & Co. Research Laboratories Indianapolis, Ind. |
| Dr. Charles W. Sensenbach | V. A. Hospital Dallas, Tex. |
| Dr. George L. Shmagranoff | Stanford U. Hospitals San Francisco, Calif. |

It would be appreciated if any member knowing the current address of any of the above members would write to: American Federation for Clinical Research, V. A. Hospital, Minneapolis 17, Minnesota.

## Obituaries

The National Office has been notified of the deaths of the following members:

Dr. Robert F. Dillon, River Forest, Illinois, November 27, 1957.

Dr. Jack D. Rosenbaum, Boston, Massachusetts, December 16, 1957.

# CURRENT COMMENT

Chairmen of the various meetings of the Federation have been invited to submit comments on any aspect of their meetings which seem to them worthy of note.

## Midwestern Section

The Midwestern Section of the American Federation for Clinical Research held its fifteenth annual meeting on October 31 in Thorne Hall on the Chicago campus of Northwestern University. Fifty-four abstracts had been submitted for consideration, of which a majority concerned general metabolism and the circulation. Several of the medical subspecialties were lightly represented. As is too often the case at Federation meetings, such major specialties like Surgery, Obstetrics and Ophthalmology were represented by only a single abstract or none at all.

Perhaps the greatest value of a medical meeting is its power to stir up ideas—thoughts which may be revealed in discussion or may be limited to inside reverberation. The extent to which this power is developed at a given session obviously depends upon many variables. A moderate amount of discussion followed most of the papers at this meeting and on one occasion some actual syntheses seemed to occur.

Tapia had described the enhanced responsiveness of patients to ganglionic blocking drugs after chlorothiazide and had shown how this was associated with a decrease of the plasma volume. There could certainly be a connection between this plasma volume fall and the known action of ganglionic blockers on venous return and cardiac output. Crosley spoke of the dual action of chlorothiazide—as an inhibitor of carbonic anhydrase, and as a blocker of renal tubular reabsorption of chloride. Rowe then dealt with the distribution of mecamylamine, noting that it enters the cells and that its excretion was less in an alkaline than in an acid urine. A discussant then noted that a decreased excretion of the blocking drugs after chlorothiazide could also explain the chlorothiazide potentiation of the blocker's effects. Other discussants noted that the clinical evidences of parasympathetic blockade seemed to increase pari passu with an increased hypotensive effect after chlorothiazide; that the increased response could be shown in one or two hours, presumably too soon for a significant drop of plasma volume to have occurred; and that

perhaps there was a direct potentiating effect dependent upon pH induced shifts of mecamylamine out of the cell. It is impossible to remember all of the discussants or precisely what was said by any one person, but the general effect was to stir up ideas.

Few comments were aroused by two papers on tolbutamide. Craig and others had found no peripheral arterio-venous glucose difference after the administration of tolbutamide into the brachial arteries of six patients. Madison and Unger had found that larger doses of tolbutamide *did* increase the peripheral arterio-venous glucose difference in dogs. They also noted the uptake of tagged insulin by the liver as splanchnic glucose output decreased. They suggested that when small amounts of insulin are released by the pancreas into the portal circulation, the amount passing through the liver and reaching the peripheral circulation may not be sufficient to produce a measurable effect. Both papers relied upon A-V differences alone, assuming blood flow to be constant.

The comments and questions which followed most of the papers not only added perspective and clarity, but kept the audience more alert and stirred up associations and ideas. The Federation should consider means of evoking *more* discussion. This should include both the fresh variety which takes shape in the mind during an interesting talk, and the spontaneous remarks which have been carefully meditated on for weeks. Basically, this is a responsibility of the entire membership.

*Robert P. Gilbert, M.D.*
*Evanston Hospital*
*Evanston, Illinois*

## Southern Section

The Southern Section of the American Federation for Clinical Research met in New Orleans, Louisiana on January 24, 1958 with over 300 members and guests present. As per custom the meeting was held in association with the Southern Society for Clinical Investigation. A joint council meeting is held each year, and this year it was voted to extend the meetings from two to two and one-half days with the first session to begin on a Thursday. The Southern Society also agreed to publish their abstracts in Clinical Research, which will be quite helpful in the mechanics of preparing the program for the two Societies.

It was gratifying that 103 abstracts were submitted to be considered for presentation. Since only 22 papers could be presented it brought up the question of having an evening session devoted to sub-sectional meetings. The present council felt that it would not suggest this change at the present time because it might tend to weaken or dilute the main meeting.

The scientific session was well attended and there was good discussion of the papers presented. The meeting was opened with a paper by a medical student. It was our impression that medical students, house officers, and research fellows should certainly be encouraged to present papers at the sectional meetings. Many excellent papers were presented and it is difficult to single out any particular one for review. The majority were well given with excellent slides and careful preparation so that the data could be presented in the allotted time.

At the business meeting Dr. Ellard Yow of Houston, Texas was elected Chairman, and Dr. John Rose of Washington, D. C. was elected Vice-Chairman. Dr. E. E. Eddleman of Birmingham, Alabama continued as Secretary for another year. The deadline for submission of abstracts was changed to November 1, 1958 to allow adequate time for submission of the program to the Editor of Clinical Research.

At the close of the scientific session the discussion was continued on a "higher plane" at a very enjoyable cocktail party.

*Kenneth R. Crispell*
*Chairman*

## Western Section

The 1958 meeting of the Western Section of the Federation was held in Carmel, California on January 29 and 30. As has been the custom for the past few years, the meeting was combined with those of the Western Association of Physicians and the Western Society for Clinical Research. Interdigitation of the sessions of the three Societies provides a pleasant change of pace during the four-day meeting and is in general happily received by the members of all groups. The Western Association of Physicians allows a leisurely presentation and discussion by senior investigators who are authorities in their field. The presentations at the Western Section of the Federation, of course, are designed primarily to allow the younger investigators in the West an opportunity to present their findings, while the Western Society for Clinical Research fills an intermediate position between these two poles.

With the gradual migration of medical research westward, the quality and number of papers submitted to these Societies has steadily improved, and the number of people in attendance has rapidly increased with each succeeding year. Not the least of the inducements to travel to Carmel in January is the physical attractiveness of the area.

Because of the increasing size of the Western Section and, in the past, its rather loose continuity of office from one year to the next, it was decided at the 1958 business meeting to change the offices in the Western Section to conform more to the pattern of other geographic Sections. Previously, only a chairman and secretary-treasurer had been elected each year. Beginning this year there will, in addition, be a vice-chairman who will ordinarily be elected to the chairmanship in the succeeding year. The officers elected for the ensuing year are Drs. Richard J. Havel of San Francisco, Chairman; Gerald T. Perkoff of Salt Lake City, Vice-Chairman; and Arno Motulsky of Seattle, Secretary-Treasurer.

One of the questions raised at the meeting of the Council, where we were fortunate in having the presence of Dr. Albert Mendeloff, National Vice President, was whether we should continue to accept funds from the pharmaceutical houses to underwrite financial support of the meetings. The Council was unanimously in favor of continuing to accept such contributions. A large part of the funds thus obtained during the present year was used to defray the expenses of the cocktail party given by the Western Section of the Federation for the members of all three Societies and their wives. This has been an annual event in the past and continues to provide an opportunity to exchange pleasantries with old and new friends which might otherwise be difficult to arrange.

Another problem discussed was whether the continuation of the various "splinter groups" should be viewed with favor or askance. These groups are growing in number each year and include the "Blood Club," "Grease Club," "Gut Club," etc. It was decided that nothing could be done to prevent or control their growth and that in reality they provided an opportunity for informal discussion which was different from anything that could be obtained within the normal framework of the programs of the various societies. It was recommended, however, that the meetings of these "clubs" should be coordinated as far as possible so that all would meet on a single night and thus leave the other evenings free for more individualized, social and scientific contact. In addition, having all such meetings at the same time would tend to keep their size within reason. Dilettantes interested in the various specialized fields would be pressed into choosing only one, rather than overcrowding a single group meeting on each successive night. Leaders of the various groups were contacted and such an arrangement was generally agreed upon.

*Monte A. Greer*
*Chairman*

## Acknowledgment

It is the primary purpose of the AMERICAN FEDERATION FOR CLINICAL RESEARCH to provide an opportunity for young investigators in the medical sciences to take part in scientific meetings and to present and publish the results of their work. In order to attain this goal, it has been necessary to maintain the dues of the organization at a modest level which does not place a burden upon men with residency or fellowship status. For years, therefore, the Federation has financed its meetings and publication in part by the generous support of various companies which manufacture drugs, pharmaceuticals and scientific apparatus.

In recognition of the valuable assistance which these concerns have given to the Federation, the Council, at its annual meeting in May, 1954, established the categories of Sponsoring, Supporting and Contributing Membership. The officers wish to take this opportunity to acknowledge the liberal help given to the Federation by the following concerns in 1958.

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## PROGRAM

# National Meeting

## American Federation for Clinical Research

**Sunday, May 4, 1958**
**Steel Pier Theater, Atlantic City, New Jersey**

**Dr. Ivan L. Bennett, Jr., Presiding**

**Presentations will be limited to ten minutes.**

MORNING SESSION

9:00–9:10—Business Meeting

1. **The Development of Diabetes Mellitus in Patients with Nondiabetic Glycosuria.**
   *Irving Paul Ackerman, Stefan S. Fajans and Jerome W. Conn.* Ann Arbor, Mich.
   *page 251*
2. **Spontaneous and Insulin-Induced Resistance of Peripheral Tissues to Insulin in Diabetes.**
   *Reubin Andres and Kenneth L. Zierler.* Baltimore.
   *page 250*
3. **Structure and Function in Diabetic Nephropathy: The Importance of Diffuse Glomerulosclerosis.**
   *Derek D. Gellman, Conrad L. Pirani, John F. Soothill, Robert C. Muehrcke, William Maduros and Robert M. Kark.* Chicago.
   *page 293*
4. **Production of Impending Hepatic Coma by Chlorothiazide and Its Prevention by Antibiotics.**
   *Joseph E. Mackie, James M. Stormont, Robert M. Hollister and Charles S. Davidson.* Boston.
   *page 301*
5. **The Effect of Potassium Repletion on the Renal-Concentrating Defect, the Renal Structural Changes, and the Cardiac and Skeletal Muscle Lesions Produced by Potassium Depletion in Rats.**
   *Walter Hollander, Jr., Robert W. Winters, John Bradley, T. Franklin Williams, William E. Loring, Jean Oliver and Louis G. Welt.* Chapel Hill, N. C. and New York City.
   *page 287*
6. **Gastrodialysis in the Treatment of Acute Renal Insufficiency.**
   *Belding H. Scribner, William R. Koreski, Thomas A. Marr and James M. Burnell.* Seattle.
   *page 295*
7. **A Search for Unsuspected Pyelonephritis among Patients with Hypertension.**
   *Hans G. Grieble, Louis C. Johnston and George Gee Jackson.* Chicago.
   *page 293*
8. **Cranberry Juice and the Antibacterial Action of Hippuric Acid.**
   *Phyllis T. Bodel, Ramzi Cotran and Edward H. Kass.* Boston.
   *page 280*
9. **Leukotoxicity of Pathologic Sera and Certain Drugs.**
   *Stuart C. Finch and Katherine D. Detre.* New Haven, Conn.
   *page 196*
10. **Pseudoxanthoma Elasticum: Clinical Findings and Identification of the Anatomic Defect.**
    *Gerald P. Rodnan, Edwin R. Fisher and Joseph E. Warren.* Pittsburgh.
    *page 236*
11. **Osteitis Deformans and Calcific Disease of the Heart Valves.**
    *Herbert Hultgren and Edward Caul.* San Francisco.
    *page 220*
12. **The Prognosis of Rheumatic Carditis.**
    *Alvan R. Feinstein and Rodolfo Di Massa.* Irvington-on-Hudson, N. Y.
    *page 220*
13. **The Oxygen Dissociation Curve in the Common Hemoglobinopathies.**
    *Theodore Rodman, Henry P. Close, William Fraimow, Richard Cathcart and May K. Purcell.* Philadelphia.
    *page 189*

# SECTION MEETINGS

## Under Joint Sponsorship of the American Federation for Clinical Research and the American Society for Clinical Investigation

## Section I

Sunday, May 4, 1958
Steel Pier Theater, 2:30 p.m.
Carolina Room, Mezzanine floor, Chalfonte, 8:00 p.m.

### Dr. James V. Warren, Chairman, Presiding

AFTERNOON SESSION

1. **A Rebreathing Method of Measuring Pulmonary Diffusing Capacity for Carbon Monoxide.**
   *Benjamin M. Lewis, Tai-hon Lin and Frances E. Noe.* Detroit. *page 312*
2. **The Effect of Altering Lung Volume on Pulmonary Diffusion of Carbon Monoxide.**
   *Birger Grapé and John M. Tyler.* Boston *page 313*
3. **Studies of Free Collapse in the Intact Human Lung.**
   *John A. Pierce.* Little Rock, Ark. *page 310*
4. **The Use of a Patient-Cycled Respirator to Evaluate Mechanical and Central Factors in the Hypercapnia of Emphysema.**
   *John M. Tyler and Birger Grapé.* Boston. (Introduced by *Thomas C. Chalmers.*) **ASCI***
5. **Effects of Rheumatoid Spondylitis on Respiration.**
   *David M. Travis, Desmond G. Julian, Charles H. Crump, Eugene D. Robin, George A. Bray, Per Helliesen and Theodore B. Bayles.* Boston. *page 318*
6. **Influence of the Rate of Coronary Plasma Flow on the Extraction of $Rb^{86}$ from Coronary Blood.**
   *W. D. Love and G. E. Burch.* New Orleans. *page 211*
7. **Measurement of Coronary Blood Flow and Myocardial Rubidium Uptake with $Rb^{86}$.**
   *David Nolting, Robert Mack, Ernst Luthy, Morton Kirsch and Charles Hogancamp.* St. Louis. (Introduced by *R. J. Bing.*) **ASCI**
8. **Systemic and Coronary Hemodynamics in Normal Men and Women.**
   *George G. Rowe, Cesar A. Castilló, George M. Maxwell, D. J. Freeman, Douglas H. White and Charles W. Crumpton.* Madison, Wis. **ASCI**
9. **Reversal of the Cardiotoxic Effects of Quinidine by Molar Sodium Lactate.**
   *Samuel Bellet, Guillermo Hamden and Andrew Somlyo.* Philadelphia. *page 220*

EVENING SESSION

1. **Left Ventricular Function at Rest and During Exercise.**
   *Carleton B. Chapman and Orland Baker.* Dallas, Tex. **ASCI**
2. **Some Factors Affecting Indicator Dilution Curves in the Presence and Absence of Valvular Incompetence.**
   *J. I. E. Hoffman and George G. Rowe.* London, England. *page 216*
3. **An Experimental Evaluation of the Indicator-Dilution Technic for the Detection of Mitral Regurgitation.**
   *Robert H. Eich, Ingolf Staib, Daniel M. Enerson and Henry Brown.* Syracuse, N. Y. *page 217*
4. **Quantitation of Valvular Regurgitation from Simultaneous Multiple Site Indicator Dilution Curves in Man.**
   *Ramon L. Lange and Hans H. Hecht.* Salt Lake City. *page 217*
5. **Relation of Size of Ventricular Septal Defects to Circulatory Dynamics.**
   *H. J. C. Swan, Roger M. Savard and John W. Kirklin.* Rochester, Minn. (Introduced by *Earl H. Wood.*) **ASCI**
6. **The Question of Vascular Hyper-responsiveness in Arterial Hypertension.**
   *Paul D. Redleaf and Louis Tobian.* Minneapolis. *page 220*

*Abstracts with "ASCI" in place of a page number will be found in the ASCI program.

# Section II

Sunday, May 4, 1958
**Vernon Room, Mezzanine floor, Haddon Hall, 2:30 p.m.**
**Viking Room, 13th floor, Haddon Hall, 8:00 p.m.**

## Dr. Laurence H. Kyle, Chairman, Presiding

### AFTERNOON SESSION

1. **Failure of Aldosterone to Maintain Sodium Retention in Normal Subjects and Addisonian Patients.**
   *Don H. Nelson and J. Thomas August.* Boston.
   **ASCI**
2. **The Metabolic Defect Responsible for Uric Acid Renal Stone Formation.**
   *Philip H. Henneman, Stanley Wallach and Eleanor F. Dempsey.* Boston. (Introduced by *A. P. Forbes.*)
   **ASCI**
3. **Alteration of Citrate Metabolism by Prednisone Therapy.**
   *Julius Mueller, Henry M. Lemon, Marcia Kelman, Joseph M. Looney and William H. Chasen.* Boston.
   *page 257*
4. **Rates of Osteogenesis Measured by Nonradioactive Strontium in Subjects with Normal and Decreased Skeletal Mass.**
   *E. Eisenberg, T. Russell Fraser and G. S. Gordan,* with the technical assistance of *Jean Marie Simien.* San Francisco, California, and London, England.
   **ASCI**
5. **Isocaloric Substitution of Carbohydrate for Dietary Protein: Effects on Serum Lipids and Lipoproteins and the Response to Androgen Administration.**
   *Robert H. Furman, R. Palmer Howard and Leonard N. Norcia.* Oklahoma City.
   *page 262*
6. **Hypothalamic Irradiation in the Rat.**
   *P. Blanquet and C. A. Tobias.* Berkeley, Calif. (Introduced by *John H. Lawrence.*)
   **ASCI**
7. **Factors Influencing Thyrotropin Metabolism in the Rat Hypophysis.**
   *John L. Bakke and Nancy Lawrence.* Seattle.
   *page 244*
8. **The Relative Influence of Maternal and Fetal Thyroid Function on Fetal and Postnatal Development.**
   *Edward A. Carr, Jr., William H. Beierwaltes, Govind Raman and Norma R. Spafford.* Ann Arbor, Mich.
   *page 243*

*To be presented if time permits:* **The Idiopathic Hypoalbuminemic Syndromes: Differentiation of Excessive Destruction from Deficient Production.**
*J. L. Steinfeld and T. Waldmann.* Bethesda, Md.
*page 209*

### EVENING SESSION

1. **Studies on Estradiol-Sensitive Isocitric Dehydrogenase in Human Breast Cancer.**
   *Vincent P. Hollander and Thelma E. Adamson.* Charlottesville, Va.
   *page 303*
2. **Norethandralone: Gonadotrophin Suppression Without Androgenic or Estrogenic Activity.**
   *Robert B. Leach, C. Alvin Paulsen, John Lanman, Norman W. Goldston and William O. Maddock.* Detroit.
   *page 261*
3. **The Metabolism of Insulin by Human Placental Tissue.**
   *Norbert Freinkel and Charles J. Goodner.* Boston.
   **ASCI**
4. **Uric Acid Riboside Phosphorylase in Human Tissues: Inhibition by Colchicine, and Other Properties.**
   *Leonard Laster and Alberta Blair.* Bethesda, Md. (Introduced by *J. E. Rall.*)
   **ASCI**
5. **Evidence for Integrity of Hypothalamic-Pituitary-Adrenal System after Steroid Withdrawal.**
   *Thomas T. Amatruda, Jr., Dorothy Hollingsworth, Nicholas D'Esopo, G. Virginia Upton and Philip K. Bondy.* New Haven, Conn.
   *page 253*

*To be presented if time permits:* **Protein Anabolism in Potassium Deficiency.**
*Euclid G. Herndon, Jr., Milton E. Rubini and William H. Meroney.* Washington, D.C.
*page 262*

## Section III

Sunday, May 4, 1958
**Viking Room, 13th floor, Haddon Hall, 2:30 p.m.**
**Rutland Room, Mezzanine floor, Haddon Hall, 8:00 p.m.**

### Dr. Arnold S. Relman, Chairman, Presiding

AFTERNOON SESSION

1. **"Low Pressure" Kidney and the Water-Concentrating Mechanism.**
   *William D. Blake.* Portland, Ore.
   **ASCI**
2. **The Relationship between Net Water Reabsorption and Osmolal Clearance as a Measure of Renal-Concentrating Activity.**
   *Lawrence G. Raisz, William Y. W. Au and Robert L. Scheer.* Syracuse, N. Y.
   *page 284*
3. **An Explanation for and Experimental Correction of the Abnormal Water Retention in Cirrhosis.**
   *Harold P. Schedl and Frederic C. Bartter.* Bethesda, Md.
   **ASCI**
4. **The Response of Tissue Electrolytes to Respiratory Acidosis.**
   *Howard Levitin, Carol R. Jockers and Franklin H. Epstein.* New Haven, Conn.
   *page 259*
5. **Renal and Cellular Responses to Acute and Chronic Respiratory Acidosis.**
   *Norman W. Carter, Donald W. Seldin and H. C. Teng.* Dallas, Tex.
   **ASCI**
6. **The Excretion of Acid in Renal Disease.**
   *Oliver Wrong and H. E. F. Davies.* Manchester, England.
   *page 290*
7. **Human Bone Electrolytes in Various Disease States.**
   *Edmund D. Pellegrino and Saul J. Farber.* New York City and Flemington, N. J.
   *page 259*
8. **Hemolysis in Uremia: Prevention of Intracorpuscular Defect by Renal Tissue.**
   *E. E. Muirhead and J. A. Stirman.* Dallas, Tex.
   **ASCI**
9. **Renal Tubular Reabsorption of Glucose and the Mechanism of Glucosuria in Pregnancy.**
   *George W. Welsh, III and Ethan A. H. Sims.* Burlington, Vt.
   *page 287*

EVENING SESSION

1. **Relationships of Hypertension and Renal Impairment to Experimental Chronic Pyelonephritis in Rats.**
   *Alvin P. Shapiro.* Pittsburgh.
   **ASCI**
2. **The Intracellular Osmolarity of Mammalian Tissues.**
   *LeRoy H. Maffly and Alexander Leaf.* Boston.
   **ASCI**
3. **An Explanation for the Apparent Nonhomogeneity of Erythrocyte Potassium Exchange.**
   *E. Raymond Borun and Seymour Perry.* Los Angeles.
   *page 188*
4. **The Electrical Potential Developed by the Large Intestine: Its Relation to Electrolyte and Water Transport.**
   *I. L. Cooperstein and Stanley K. Brockman.* Bethesda, Md.
   *page 277*
5. **Effects of Calcium on Urine-Concentrating Ability.**
   *Jacob Grossman, Martin F. Mines, Arthur G. Goldman and Morris Wolfman.* New York City.
   **ASCI**

## Section IV

Sunday, May 4, 1958

**Carolina Room, Mezzanine floor, Chalfonte, 2:30–5:30 p.m.**

### Dr. Lawrence E. Young, Chairman, Presiding

1. **Investigations of the Mechanisms of Thrombocytopenia.**
   *Frank H. Gardner, Knut A. Aas, Phin Cohen and James C. Pringle.* Boston.
   *page 199*
2. **Leukokinetics as Measured with Radioactive Diisopropylfluorophosphate ($DFP^{32}$).**
   *John W. Athens, Alvin M. Mauer, Helen Ashenbrucker and George E. Cartwright.* Salt Lake City.
   ASCI
3. **Anticomplementary Activity of Multiple Myeloma.**
   *Marvin L. Bloom, Sidney Shulman and Ernest Witebsky.* Buffalo.
   *page 206*
4. **The Hemopoietic Effects of Batyl Alcohol.**
   *James W. Linman.* Chicago. (Introduced by *Frank H. Bethell.*)
   ASCI
5. **Sequestration of Reticulocytes and of Abnormal Red Cells by Filtration at Low Pressures.**
   *James H. Jandl.* Boston.
   ASCI
6. **Erythrocyte Survival and Heme Synthesis in Lead Poisoning.**
   *Robert C. Griggs and John W. Harris.* Cleveland.
   *page 188*
7. **Compensatory Mechanisms in Primaquine-Sensitive Erythrocytes.**
   *Stanley L. Schrier and Robert W. Kellermeyer.* Chicago. (Introduced by *Alf S. Alving.*) ASCI
8. **Homografts of Bone Marrow in Dogs after Lethal Total-Body Radiation.**
   *E. Donnall Thomas, Harry L. Lochte, Jr., Alfred Jaretzki III, Charles A. Ashley and Otto D. Sahler.* Cooperstown, N. Y. (Introduced by *Joseph W. Ferrebee.*)
   ASCI

## Section V

Sunday, May 4, 1958

**Viking Room, 13th floor, Haddon Hall, 2:30–5:30 p.m.**

### Dr. Samuel P. Martin, Chairman, Presiding

1. **Studies in Human Ecology: Perceptions of Life Experiences as a Determinant of the Occurrence of Illness.**
   *William N. Christenson, Francis D. Kane, Harold G. Wolff and Lawrence E. Hinkle, Jr.* New York City. *page 238*
2. **Clinical and Pathologic Studies in Severe Asian Influenza Infections.**
   *Fred R. McCrumb, Jr., Paul F. Guerin, George K. Baer and Theodore E. Woodward.* Baltimore. *page 282*
3. **Chronic Salivary Gland Virus Infection in Children.**
   *Wallace P. Rowe.* Bethesda, Md.
   ASCI
4. **Susceptibility of Rats with Hormonal Hypertension to Experimental Pyelonephritis.**
   *James W. Woods.* Chapel Hill, N. C.
   *page 228*
5. **The Febrile Response upon Injection of Bovine Albumin into Previously Sensitized Rabbits.**
   *Richard Studley Farr.* Pittsburgh.
   ASCI
6. **Cross Resistance to Cerebral Typhoid Infection and Influenza Virus Neurotoxicity.**
   *Edward W. Hook and Robert R. Wagner.* Baltimore. ASCI
7. **Prevention of Rubella with Gamma Globulin.**
   *Harold B. Houser and Norbert Schalet.* Syracuse, N. Y. *page 281*
8. **Hemadsorption in the Diagnosis and Study of Viral Infections.**
   *Alexis Shelokov.* Bethesda, Md. ASCI

## Section VI

Sunday, May 4, 1958
West Room, 13th floor, Haddon Hall, 2.30 p.m.

### Dr. Albert I. Mendeloff, Chairman, Presiding

1. **Changes in Wedged Hepatic Venous Pressure and Hepatic Blood Flow Accompanying Clinical Improvement in Cirrhosis.**
   *Telfer B. Reynolds, Alan G. Redeker and Herman M. Geller.* Los Angeles. ASCI
2. **Clinical Studies with Penicillamine in Hepatolenticular Degeneration.**
   *Marvin J. Seven, Bernard Kliman and Ralph E. Peterson.* Bethesda, Md.
   *page 302*
3. **The Capacity for Bilirubin Production as Reflected by the Concentration of Plasma Bilirubin.**
   *William H. Crosby.* Washington, D.C.
   ASCI
4. **Studies of Glucuronide Synthesis and of Glucuronyl Transferase Activity in Liver and Serum.**
   *Irwin M. Arias, Bertram A. Lowy and Irving M. London.* New York City. ASCI
5. **Islet Cell Tumor and a Syndrome of Refractory Watery Diarrhea and Hypokalemia.**
   *John V. Verner and Ashton B. Morrison.* Durham, N. C. *page 274*
6. **The Association of Peptic Ulcer and Hereditary Hyperparathyroidism.**
   *Charles E. Jackson.* Bluffton, Ind.
   *page 272*
7. **Comparative Effect of Parasympathomimetic Agents and 5 Hydroxytryptamine upon Colon Contractility in Dogs.**
   *Marvin H. Sleisenger, David H. Law, Charles M. Lewis and James H. Pert.* New York City.
   *page 276*
8. **Direct Measurement of Cholesterol Absorption via the Thoracic Duct in Man.**
   *Leon Hellman, E. L. Frazell and R. S. Rosenfeld.* New York City.
   ASCI
9. **A Quantitative Comparison of Indices of Malabsorption: $I^{131}$ Triolein, Fat and Nitrogen Balance, Glucose and Vitamin A Absorption Curves.**
   *Arthur B. French, Makoto Ishikawa, Hugh S. Wiggins and H. Marvin Pollard.* Ann Arbor, Mich.
   *page 276*

## Section VII

Sunday, May 4, 1958
West Room, 13th floor, Haddon Hall, 8:00 p.m.

### Dr. Robert R. Wagner, Chairman, Presiding

1. **Evaluation of a Medical Student Research Program.**
   *William P. Nelson, III.* Albany, N. Y.
   *page 240*
2. **The Honors Program of the New York University College of Medicine.**
   *Chandler A. Stetson, Jr.* New York City.
   *page 241*
3. **Stimulation of Student Interest in Research by a Tutorial Program.**
   *Claude A. Villee.* Boston.
   *page 241*
4. **The Rochester Student Fellowship Program.**
   *Leonard D. Fenninger.* Rochester, N. Y.
   *page 241*
5. **Research as Part of the Medical Curriculum.**
   *Vernon W. Lippard and Arthur Ebbert, Jr.* New Haven, Conn. *page 240*

# Advance Research Reports Submitted to the Annual NATIONAL MEETING of the American Federation for Clinical Research

Atlantic City, New Jersey · Sunday, May 4, 1958



## BLOOD

### Standard Metabolic Rate and Circulating Red Cell Volume


By *A. Contopoulos.* Donner Laboratory and Institute of Experimental Biology, University of California, Berkeley.


*(This abstract withdrawn by request of the author.)*

### Erythrocyte Magnesium in Health and Disease


By *William O. Smith, Raoul Carubelli and James F. Hammarsten.* Medical and Radioisotope Services, Oklahoma City V. A. Hospital, and

Department of Medicine, University of Oklahoma School of Medicine.

The clinical syndrome of magnesium deficiency has been observed in patients with and without a low serum magnesium. It has been postulated that the intracellular magnesium concentration is more important in determining clinical symptomatology.

Erythrocytes were selected for study of intracellular magnesium because of their availability. A method for determination of erythrocyte magnesium has been developed in our laboratory, based on a modification of the EDTA titration method for calcium and magnesium described by Zak and co-workers.

Erythrocyte magnesium was studied in 13 healthy volunteers and a mean value of 5.29 ± 0.34 mEq./L. was obtained. Plasma magnesium was 1.80 ± 0.13 mEq./L., giving a $\frac{\text{RBC}}{\text{Plasma}}$ ratio of 2.95 ± 0.32.

A small group of patients have been studied to date. In 4 patients with delirium tremens erythrocyte magnesium was 2.30 ± 0.70 mEq./L. All patients had abnormally low values. The erythrocyte magnesiums were relatively much lower than the plasma values. Erythrocyte magnesium in 6 patients with uremia and CNS depression was 8.91 ± 1.48 mEq./L. All patients had obnormally high values. Two had normal plasma magnesium levels.

### Linkage Between Triose Phosphate Metabolism and Potassium Flux in the Human Erythrocyte

By *Searle B. Rees, Thomas J. McManus, William L. McLellan, Fabian J. Lionetti, John G. Gibson, II and John P. Merrill.* Medical Service, Peter Bent Brigham Hospital, and Department of Medicine, Harvard Medical School, Boston.

We have shown that the nucleoside, inosine, is superior to dextrose as an energy source in vivo and in vitro for reaccumulation of erythrocyte phosphate esters and potassium in certain depleted pathologic states. Specific enzymatic assays of cell adenine nucleotides and resin chromatography of the key intermediates of the glycolytic and pentose phosphate pathways have been studied with simultaneous measurements of potassium[42] flux under a variety of controlled conditions in vitro.

$10^{-6}$ M of ouabain/L. RBC produce 75–80% inhibition of potassium influx without impairing phosphate or carbohydrate metabolism. Thus, the transfer of inorganic phosphate and pathways of carbohydrate metabolism are not linked in a compensatory fashion to an "active" potassium influx. However, a comparable 75–80% inhibition of potassium influx can be produced by blocking triosephosphate dehydrogenation with $10^{-3}$ M of sodium iodoacetate (IAA) per liter of RBC. Nucleoside phosphorolysis and the pentose phosphate pathway are not inhibited by $10^{-3}$ M IAA. An erythrocyte ghost preparation depleted of diphosphopyridine nucleotide can also metabolize inosine to hexose- and triosephosphate, but is unable to accumulate or actively transport potassium.

The phosphate esters which accumulate in the presence of IAA apparently leak from the erythrocyte. This leakage of ester phosphate is associated with a significant increase in potassium efflux. These studies suggest that triosephosphate metabolism is the final common pathway for regeneration of high energy phosphate and maintenance of an "active," glycoside-sensitive, potassium influx in the human erythrocyte.

### Studies of the Lipids of Normal and Stored Human Red Cells

By *Claude F. Reed, Eva G. Eden and Scott N. Swisher.* University of Rochester School of Medicine and Dentistry, Rochester, New York.

Using quantitative chromatographic technics developed at this institution, we have studied the lipid composition of the red blood cells from normal human males and females between the 2nd and 8th decades. The total lipid content of the normal human red cell is 4.95 Gm. × $10^{-13}$ ± 0.26 (2 S.D.) per cell. This includes phospholipids (65–72%), cholesterol (22%) and traces of neutral fat, free fatty acids and cholesterol esters, totalling less than 6%.

The lipid phosphorous is partitioned as follows: sphingomyelin (22%), lecithin (30%), phosphatidyl serine ((15%), phosphatidyl ethanolamine (25%), and about 2.5% each of lysolecithin, inositol phosphatide and phosphatidic acid.

We have studied the lipid composition of normal red blood cells stored in glass, in ACD formula A, at 4 C. During the first 21 days of storage a steady and equal decrease in the total lipid, lipid phosphorous, and cholesterol (about 22% in each) per cell was found. All the phospholipids were lost in proportion to their initial concentration. On serial determinations between the 21st and 42nd days of storage, no further

changes in lipid content could be demonstrated. These data suggest that loss of one or more labile lipid fractions may be related to rapid loss of viability in cells stored beyond 21 days.

Normal human red cells incorporated very small amounts of $C^{14}$-labeled acetate into the lipid fraction upon in vitro incubation. After five days of storage, the red cells no longer incorporated $C^{14}$ into lipids. In vitro incorporation of $P^{32}$ orthophosphate was primarily into phosphatidic acid and was unchanged by storage for 21 days.

### The Effect of Anemic Anoxia on the Cellular Development of Nucleated Red Cells

By *Allan J. Erslev.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston. (Aided by a research grant from the National Institutes of Health.

It is generally accepted that normal red cell production is regulated by a feedback mechanism which balances the demand for oxygen in the tissues with the production of oxygen-carrying hemoglobin.

The dynamics of this regulatory mechanism are poorly understood, but it appears that a hypothetical anoxic stimulus is transmitted from a specific oxygen-sensitive tissue to the bone marrow by means of a humoral factor. In the marrow the resulting increase in red cell production may be due either to (a) an increase in the rate of differentiation of multipotential "stem" cell into pronormoblasts or (b) an acceleration of the rate of division of already formed nucleated red cells.

In order to distinguish between these alternatives, normal rabbits were bled 20 ml./Kg., kept anemic for 20 hours and then reinfused with the previously removed blood. A characteristic reticulocyte response began after one day and was maximal after 3 days, despite the absence of sustained anemic anoxia. When mitotic division was abolished immediately after the period of anemic anoxia by means of colchicine or nitrogen mustard, the reticulocyte response began as usual, but disappeared completely 24 hours later. However, when bone marrow activity was suppressed, immediately after the period of anemic anoxia, for 4 days by an atmosphere containing 65% oxygen, an unmodified, characteristic reticulocyte response was observed. Likewise, when mitotic division was arrested by colchicine during the period of anemic anoxia, the onset of the reticulocyte response, though delayed by 2 days, was otherwise of characteristic magnitude.

The conclusions drawn from these observations are: (1) The erythropoietic response to anoxia is mediated in the bone marrow by changing undifferentiated "stem" cells into differentiated pronormoblasts; (2) the maturation and multiplication of differentiated red cells takes place at a fixed rate, is independent of the anoxic stimulus and is solely dependent on an adequate nutritional and hormonal environment.

### Glucose Metabolism and Oxygen Consumption in Normal and Glucose-6-phosphate Dehydrogenase Deficient Human Erythrocytes

By *Anne B. Johnson and Paul A. Marks.* Columbia University, College of Physicians and Surgeons, New York.

The relationships between glucose-6-phosphate dehydrogenase (G-6-P.D.) activity, $O_2$ consumption, and $CO_2$ formation were studied in mature human erythrocytes (RBC) with normal and reduced G-6-P.D. levels. The latter cells included old RBC (previously reported to have a reduction in G-6-P.D.) and RBC with a marked hereditary deficiency of this enzyme.

Normal RBC appear to oxidize glucose to $CO_2$ only by reactions presumably requiring G-6-P.D., involving the oxidative decarboxylation of carbon-1 of glucose. This is indicated by a rate of $C^{14}O_2$ production from 1-$C^{14}$-glucose 200 times that from 6-$C^{14}$-glucose. This finding is consistent with previous observations that certain Krebs cycle enzymes are lacking in mature RBC.

In studies with normal RBC, the mean values were: G-6-P. D., 17.3 units ($\Delta$ O.D./min./Gm. Hgb.); $O_2$ consumption (in the presence of methylene blue) 574 $\mu$L./hr./Gm. Hgb.; and $C^{14}O_2$ yield from 1-$C^{14}$-glucose, 12%/Gm. Hgb. In RBC with hereditary G-6-P. D. deficiency, the average enzyme activity was only 17%; $O_2$ consumption, 35%; and $C^{14}O_2$ yield from 1-$C^{14}$-glucose, 57% of normal RBC values.

In young compared to old RBC, the G-6-P. D. levels were 19.1 and 16.0 units, and $O_2$ consumptions were 529 and 370 $\mu$L./hr./Gm. Hgb., respectively. In contrast, similar fractionation of RBC with hereditary G-6-P. D. deficiency revealed that young compared to old cells had G-6-P. D. levels of 11.4 and 2.2 units, and

$O_2$ consumptions of 223 and 47 μL./hr./Gm. Hgb., respectively.

RBC with hereditary deficiency of G-6-P. D. had normal levels of adenosine triphosphate and of the enzymes 6-phosphogluconic dehydrogenase, phosphohexose isomerase and lactic dehydrogenase.

These data indicate that a decrease in G-6-P. D. activity in mature RBC is associated with a parallel reduction in $O_2$ consumption and $CO_2$ production from carbon-1 of glucose.

### An Explanation for the Apparent Nonhomogeneity of Erythrocyte Potassium Exchange

By *E. Raymond Borun and Seymour Perry.* Wadsworth V. A. Center, and University of California Medical Center, Los Angeles.

Solomon and Gold have suggested that the kinetics of erythrocyte potassium exchange can be best explained by assuming that there is a rapidly and a slowly exchanging fraction of cell potassium. These fractions might be present in different physical locations in each cell, in different chemical combinations, or in different cell populations. Harris and Prankard recently presented an anlysis based on the concept that the potassium fractions occupy different locations in the cell. The present data, however, indicate that different cell populations are responsible for the nonhomogeneity of erythrocyte potassium.

As an extension of a recent study which demonstrated that ageing erythrocytes are concentrated in the bottom layer of centrifuged blood specimens, $K^{42}$ uptake and relative specific activity were determined in the top, middle and bottom layers of erythrocytes in centrifuged blood specimens from 12 subjects. Erythrocyte counts in quadruplicate, dry weight, and trapped plasma ($I^{131}$ serum albumin) were determined in the erythrocyte layers of 12 other specimens.

Relative specific activity was 27 ± 2% (S.E.) higher in the bottom than in the top layer after two hours incubation with $K^{42}$, thus localizing the rapidly equilibrating potassium to the cells of this layer. $K^{42}$ uptake and potassium exchange per liter of erythrocytes were significantly higher in the bottom layer (14 ± 1% and 17 ± 1% respectively); however, the mean uptake and exchange calculated per unit cell were similar in the two layers. The more rapid equilibration of potassium in the old cells of the bottom layer, despite similar $K^{42}$ uptake per unit cell in both layers, can largely be explained by the observation that there is 13 ± 2% less water and 22% less potassium per unit cell in the bottom than in the top layer.

### Erythrocyte Survival and Heme Synthesis in Lead Poisoning

By *Robert C. Griggs and John W. Harris.* Medical Service, Cuyahoga County Hospital, Cleveland, and Department of Medicine, Western Reserve University School of Medicine.

The survival time of autotransfused erythrocytes, tagged with $Cr^{51}$, was observed in 4 adult males who had lead intoxication resulting from industrial exposure. The half-life of the erythrocytes was observed to be 20, 20, 25, and 26 days compared to a normal minimum of 30 days. Body-surface scanning for radioactivity did not indicate any preferential organ accumulation as an indication of erythrocyte sequestration.

Quantitative determinations of the urinary excretion of the heme precursors, delta-aminolevulinic acid (δAL) and porphobilinogen (PBG), were made for patients with clinical and laboratory evidences of lead toxicity and for workers with histories of lead toxicity, or recent exposure to lead. Although the excretion of PBG was consistently normal, δAL excretion was markedly increased in individuals with lead poisoning and moderately increased in individuals with histories of lead intoxication or exposure (even though the excretion of coproporphyrin was not increased above normal). The excretion of δAL temporarily decreased to near normal levels following the administration of versene i.v., but elevated urinary levels persisted for months after the cessation of toxic manifestations and despite the absences of subsequent exposure to lead. In contrast, in 3 patients with acute intermittent porphyria the excretion of both δAL and PBG was markedly elevated.

These studies provide additional evidence that heme synthesis is altered and that the rate of erythrocyte destruction is accelerated in lead poisoning. The determination of urinary delta-aminolevulinic acid appears to be a sensitive test to aid in detection or exclusion of lead intoxication.

### The Influence of Diet on the Distribution of Iron in the Rat Liver

By *Masafumi Seki and Thomas C. Chalmers.* Lemuel Shattuck Hospital and Harvard Medical School, Boston.

Despite the prevalent belief that cirrhosis

of the liver in hemochromatosis results from the toxic effects of excessive iron absorption, the disease has not been reproduced by iron administration in animals. To test the alternate hypothesis that coexistent liver disease affects iron metabolism, 3 groups of 6 rats each were fed a normal diet for 6 months and 3 groups, an 8% protein, low choline diet. At each protein level the diets contained 0.06, 3 and 6% ferric citrate.

In addition to H and E and iron stains and chemical determination of nonhemin iron, the perfused livers were homogenated in 0.1 M phosphate buffer, pH 7.0, and fractionated as follows: $P_1$, precipitate obtained by heating at 80 C. for 10 minutes (? hemosiderin); $P_2$, obtained by heating supernatant of $P_1$ for 30 minutes at 100° (ferritin); $P_3$, supernatant of $P_2$ heated at pH 4.0 and 100° for 30 minutes (other high molecular compounds).

None of the rats had cirrhosis, but the low protein group had varying degrees of central necrosis. Distribution of iron was distinctly different, concentrated in the portal areas in the normal diet group and diffuse in the low protein group.

The mean total nonhemin iron at the 3 levels of dietary iron were 144, 449, and 1565 on the normal diet, and 154, 950, and 2478 μg./Gm. wet weight on the low protein diet. The ratios of $P_2$ to $P_1$ respectively were 0.49, 0.35, and 0.21 on the normal diet, and 0.71, 0.46, and 0.37 on the low protein diet. Twenty-four hours after injection of $Fe^{59}$, $P_2$ had almost twice the specific activity of $P_1$ in all groups.

It is suggested that in rats on a deficient diet a distinctly different histologic and biochemical distribution of excessively absorbed iron is related to the coexistent liver disease.

### Separation of Hemoglobins by Starch Gel Zone Electrophoresis

By *Ralph L. Engle, Jr., Ann Markey, James H. Pert and Kenneth R. Woods.* Department of Medicine, New York Hospital–Cornell Medical Center, New York.

Starch gel zone electrophoresis (Smithies) was used to separate hemoglobins to see if it had advantages over conventional filter paper methods. Hemoglobin solutions were made by washing red blood cells with saline, lysing with water-toluene, and removing the red cell membranes by centrifugation. Gels were formed by heating hydrolyzed potato starch with borate buffer .03M pH 9.05. Hemoglobin was applied by inserting a small, rectangular piece of filter paper saturated with the sample into the gel. A potential of 4.5 v./cm. was applied for 16 hours at 10 C. The strips were examined before and after staining with a protein stain, napthalene black B 200.

In hemoglobin preparations from normal individuals it was possible to identify 3 pigmented ($A_o$, $A_1$, $A_2$) and 2 nonpigmented ($a_o$, $a_1$) bands in order from the cathodic end. The $a_1$ band migrated toward the anode. The principal band was the $A_1$ band. In hemoglobin from cord blood, the bands in order from anode to cathode were $A_o$, $A_1$, F. Hemoglobin F was easily distinguished from hemoglobin $A_1$. Bands $A_2$, $a_o$ and $a_1$ were absent. Bands identified in the various hemoglobin abnormalities were as follows (from anode to cathode): (1) Thalassemia major, $A_o$, $A_1$, F, $A_2$, $a_o$, $a_1$; (2) Thalassemia minor, $A_o$, $A_1$, $A_2$, $a_o$, $a_1$; (3) Sickle trait, $A_o$, $A_1$, ?F, S, $A_2$, $a_o$, $a_1$; (4) Sickle cell anemia, $A_o$, F, S, $A_2$, $a_o$, $a_1$; (5) Thalassemia–sickle cell disease, $A_o$, F, S, $A_2$, $a_o$, $a_1$; (6) Hemoglobin C- sickle cell disease, $A_o$, F, S(C-$A_2$) $a_o$, $a_1$; and (7) Hemoglobin H trait, H($A_o$ or $A_1$) $a_o$, $a_1$. Hemoglobin C migrated in same region as hemoglobin $A_2$.

The resolving power achieved by zone electrophoresis of hemoglobins in starch gels was superior to that obtained with the use of filter paper. Additional pigmented and nonpigmented bands were identified. Fetal hemoglobin was readily and distinctly separated from normal hemoglobin.

### The Oxygen Dissociation Curve in the Common Hemoglobinopathies

By *Theodore Rodman, Henry P. Close, William Fraimow, Richard Cathcart and May K. Purcell.* V. A. Hospital, and Jefferson Medical College, Philadelphia.

The presence of arterial oxygen unsaturation has been reported in sickle cell anemia. This has usually been attributed to pulmonary lesions accompanying the disease. To explore the etiology of this unsaturation, complete pulmonary function studies were performed on a group of subjects who had either sickle cell anemia, S-C hemoglobin disease, the sickling trait, or the C hemoglobin trait. The hemoglobin pattern of these subjects was electrophoretically determined. In addition to the usual tests of ventilation, distribution and diffusion, the physiologic oxygen

dissociation curves were plotted. This was accomplished by allowing the subject to breathe various concentrations of oxygen and determining simultaneously the oxygen saturation and the partial pressure of oxygen in the arterial blood. In this way the physiologic dissociation curve for each subject could be determined.

No significant aberration of pulmonary function was found in any of the subjects studied. The presence of arterial oxygen unsaturation in sickle cell anemia was confirmed. A tendency toward unsaturation was found in hemoglobin S-C disease. The failure to saturate was due to a marked displacement of the oxygen dissociation curve to the right of normal. Our findings suggest that in some patients with hemoglobin defects, oxygen saturation of the arterial blood cannot be achieved under physiologic conditions though no lung disease is present. The transfusion of 1500 cc. of normal blood into one subject with sickle cell anemia shifted his dissociation curve towards normal. The dissociation curve was normal in subjects who had predominantly A hemoglobin with small quantities of S or C hemoglobin.

The mechanism of the abnormality that we have described is not apparent. One possibility is that the abnormal hemoglobin may be associated with a reduction in the pH of the red cell which is responsible for the displacement of the dissociation curve.

### The Effect of Hypoxia in Patients with Sickle Cell Trait

By *William C. Levin, G. W. N. Eggers, Jr. and John E. Perry.* Departments of Internal Medicine and Anesthesiology, and Hematology Research Laboratory, University of Texas Medical Branch, Galveston. (Aided by a United States Air Force Research contract.)

Patients with AS hemoglobinopathy were subjected to hypoxia by having them breathe mixtures of nitrogen and oxygen. Relative concentrations of these gases were adjusted to simulate the oxygen content of the atmosphere at altitudes of 5,000, 7,000 and 8,000 feet. Autologous erythrocytes labeled with radioactive chromate were injected at the beginning of the hypoxic period. Activity in the spleen and liver was subsequently measured and erythrocyte survival time was determined.

In normal individuals (hemoglobin AA genotype) hypoxia produced no change in splenic or hepatic concentration of labeled erythrocytes and did not shorten erythrocyte survival time. In patients with sickle cell trait (hemoglobin AS genotype), alterations of these parameters were noted neither in the absence of hypoxia nor with hypoxia simulating an altitude of 5,000 feet. However, with hypoxia simulating altitudes of 7,000 feet and 8,000 feet, there occurred hypersequestration of the labeled erythrocytes in the spleen and shortening of erythrocyte survival time. Both of these alterations were usually transient, persisting for 3 to 5 days. Cross transfusion experiments were performed in which 2 normal volunteers were subjected to hypoxia following the intravenous administration of $Cr^{51}$-labeled compatible erythrocytes from a donor with sickle cell trait. In one of these recipients, there was moderate reduction of erythrocyte survival time without evidence of splenic hypersequestration, while in the other volunteer no alteration from normal was noted.

These observations support the concept that individuals with AS hemoglobinopathy may develop thrombotic and/or hemolytic disease if they are exposed to sustained hypoxia.

### The Nephropathies of Sickle Cell Disease, A Case Study

By *Leonard B. Berman.* Renal Laboratory, Georgetown Division, D. C. General Hospital, Washington, D. C.

An 8-year-old Negro male with homozygous hemoglobin S recently presented with many, if not all, of the presently known renal complications of sickle cell disease. Clinical studies combined with renal biopsy revealed the following combination of nephropathies: nephrotic syndrome, a Pitressin-resistant concentrating defect, pyelonephritis, miliary infarcts and gross hematuria.

Originally referred because of proteinuria, the patient was found to excrete 17 Gm./day along with doubly refractile fat bodies. Hypoalbuminemia and hypercholesterolemia were present. Biopsy revealed some glomeruli solidly packed with sickled cells. Other glomeruli showed a focal hyalinization. The urinary electrolyte pattern was unusual for a nephrotic patient in that urine sodium concentrations consistently exceeded 65 mEq./L. The absence of edema may have been related to the rate of sodium excretion. Two weeks of ACTH therapy produced no significant changes.

Urinary specific gravities on random specimens declined steadily with increasing years

1.012 was the highest recorded since age 6. The patient's ability to maximally concentrate urine was tested both by prolonged dehydration and by the administration of Pitressin during a water diuresis. The urine to plasma osmolar concentration ratio never rose above 1.4. Since renal function, as measured by endogenous creatinine clearance and PSP excretion, was otherwise excellent, this defect in concentrating power may be specifically related to sickle cell disease as described by other investigators. ACTH administration did not change the Pitressin resistance. The effect of multiple transfusions is under study.

The biopsy also revealed widespread pyelonephritis and miliary infarcts. The first of these was accompanied only by asymptomatic pyuria.

A history of gross hematuria was obtained but this did not recur under observation. The most likely areas of bleeding, i.e. the papillae, were not included in the biopsy specimen.

### The Mechanism of Action of Thrombin Preparations on Hemolysis of Paroxysmal Nocturnal Hemoglobinuria Erythrocytes

By *Carl F. Hinz, Jr.* Department of Medicine, Western Reserve University School of Medicine, and University Hospitals, Cleveland.

The hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH) by normal human serum in vitro is increased by the addition of preparations of bovine thrombin. Addition of thrombin to serum does not hemolyze normal cells. This effect on PNB hemolysis has been attributed to the clotting properties of the thrombin preparations, but the present studies indicate that it is due to antibodies to human red cells which are contained in the thrombin preparations. PNH cells are known to be hemolyzed by amounts of antibody that cause only agglutination of normal erythrocytes.

Five preparations of bovine thrombin were tested. None contained properdin activity, but all agglutinated human erythrocytes. Although hemolysis of PNH cells by normal serum occurs optimally at acid pH and no agglutination occurs, addition of 50 units of thrombin to serum resulted in marked hemolysis and agglutination of PNH cells at both acid and alkaline pH. Heating the thrombin preparations at 56 C. for 30 minutes almost completely inactivated thrombic activity for clotting, but did not alter the agglutinating or hemolytic property for PNH cells. Adsorbtion of thrombin preparations with human erythrocytes reduced the agglutinating and hemolytic activity for PNH cells, but did not alter significantly the thrombic activity for clotting.

A preparation of thrombin made from human plasma contained isoagglutinins. When added to normal serum it caused agglutination and increased the hemolysis of PNH cells of group A, but did not affect PNH cells of group O. The effect on agglutination and hemolysis of PNH cells of group A was diminished by adsorbing the thrombin preparation with normal group A erythrocytes or by adding A substance. Thrombin activity for clotting was unaltered by these procedures.

Thus, the effect of thrombin preparations on PNH hemolysis is probably due to heterophile or iso-antibody to red cells contained in thrombin preparations.

### Erythrocyte Kinetics in Patients with Polycythemia Vera

By *Myron Pollycove and John H. Lawrence.* Donner Laboratory, University of California, Berkeley.

Considerable controversy exists regarding the relationship of myeloid metaplasia to polycythemia vera. There is also uncertainty about erythrocyte lifespan and pathogenesis of anemia in patients with polycythemia vera. These aspects were investigated as follows:

Thirty-six patients with polycythemia vera, previously observed for up to 20 years, and 12 normal subjects were studied by injection of plasma-bound radioiron. Red cell volume was determined with $P^{32}$-labeled erythrocytes. Daily hemoglobin synthesis was determined by plasma radioiron analysis for 10 or more days. Mean erythrocyte lifespan was determined by relating hemoglobin synthesis to total body hemoglobin. Splenic sequestration and destruction of erythrocytes and extramedullary erythropoiesis were measured with body surface scintillation counters.

Patients were grouped according to erythrocyte kinetic patterns. Those with normal erythrocyte lifespan and marrow erythropoiesis comprise Group I; shortened erythrocyte lifespan with splenic sequestration and destruction of erythrocytes, Group II; and extramedullary erythropoiesis (myeloid metaplasia), Group III. Mean values for Groups I (11 patients), II (9 patients), and III (17 patients), respectively, are: Hb concentration–19.2, 17.4, 12.6 Gm./100 ml. [normal 15.1]; total body Hb–19.0, 15.4, 11.9 Gm./Kg. [normal 10.6]; daily Hb synthesis–1.6,

2.9, 3.6 Gm./L. [normal 1.3]; mean erythrocyte lifespan—116, 63, 45 days [normal 117]; time elapsed from initial diagnosis to study—2.1, 4.7, 9.7 years; blood volume—99, 87, 95 ml./Kg. of body weight [normal 70]. One patient in Group II, restudied 5 years later, had progressed into Group III. Seven patients in Group III had been carefully observed for 3–19 years before nucleated erythrocytes appeared in circulating blood. One patient in Group III was untreated except for two 500 ml. venesections 18 years previously.

These results suggest that in the natural history of polycythemia vera, patients proceed through 3 stages corresponding to the 3 groups studied.

### Urinary 17-Ketosteroids and 17-Hydroxycorticoids in Polycythemia

By *Franklin E. Walker, John H. Lawrence and Franco F. Sangalli.* Donner Laboratory and Donner Pavilion, University of California, Berkeley.

We have previously described a group of patients having what we have called the polycythemia of stress, characterized by an elevated red cell count but a normal total red cell volume and a low plasma volume. The cause of the hemoconcentration is unknown. Obesity is present in about 50% of these patients, and hypertension of varying severity is frequently observed. None of the patients studied showed definite clinical evidence of increased adrenocortical function. The majority exhibit symptoms of mental stress, anxiety or mild psychoneurosis.

A study of the patterns of urinary excretion of 17-ketosteroids and 17-hydroxycorticoids in patients with this form of relative polycythemia and with polycythemia vera is presented. The 17-ketosteroids and 17-hydroxycorticoids in 24-hour urine samples were measured by the Callow modification of the Zimmerman procedure and the Norymberski sodium bismuthate oxidation method, respectively. Both measurements were made concurrently on the same sample. The normal values obtained by these methods are as follows: 17-ketosteroids, 3–20 mg./24 hours, and 17-hydroxycorticoids, 3–12 mg./24 hours. Patients with polycythemia vera (16 patients, 20 samples) showed a mean 24-hour urinary excretion of 7.45 mg. of 17-ketosteroids and 6.76 mg. of 17-hydroxycorticoids, while patients with relative polycythemia of stress (12 patients, 16 samples) showed a mean 24-hour excretion of 17.96 for the 17-ketosteroids and 20.32 mg. for the 17-hydroxycorticoids.

These findings would suggest that adrenal cortical activity is increased in patients presenting polycythemia of stress. The reasons for this increase are not clear. The observation of increased 17-hydroxycorticoid excretion in severely psychotic patients would suggest that mental stress could be a significant factor in the increased 17-hydroxycorticoid levels observed in these patients.

### Variability of Intrinsic Factor Activity in Pernicious Anemia

By *George O. Clifford and Georgia A. Lewis.* Department of Medicine, Wayne State University College of Medicine, and City of Detroit Receiving Hospital.

It is currently accepted that vitamin $B_{12}$ absorption is conditional upon the presence of intrinsic factor in the stomach and that this factor must be absent for years before pernicious anemia develops. Forty-eight patients diagnosed as having pernicious anemia were studied by means of the Schilling test ($Co^{60}$ $B_{12}$) for possible variation in absorption of the labeled vitamin. All patients had presented with megaloblastic anemia in relapse from 18 years to 1 week previously. Pernicious anemia was considered established only if the initial $Co^{60}$ $B_{12}$ excretion was lower than 4% and excretion was enhanced to greater than 8% by addition of intrinsic factor. Schilling tests were repeated at 3-month intervals for 9–12 months.

Thirty-three patients fulfilled the above criteria for pernicious anemia, $B_{12}$ excretion remaining consistently reduced during the study. Duration of the disease and condition at time of study did not influence the constancy of results.

Eight patients with low initial $B_{12}$ excretion experienced no increased excretion with intrinsic factor and were considered to have megaloblastic anemia of some other etiology. Renal disease was excluded in this group.

Five patients revealed normal excretion values indicating adequate intrinsic factor and excluding classical pernicious anemia.

Two intriguing patients exhibited initial low excretion of $Co^{60}$ $B_{12}$ which increased to normal with intrinsic factor. Subsequent values, however, varied between the normal and low-normal range. This implied a spontaneous variation in intrinsic factor activity in these patients.

The data generally confirm the reliability of the Schilling technic in the diagnosis of pernicious anemia and related syndromes. An occasional patient may be found, however, in whom variable intrinsic factor activity may be detected by this procedure. Spontaneous remissions infrequently observed prior to liver therapy may have been on such a basis.

### Erythrocytosis after Unilateral Partial Ligation of Renal Vein in Dogs

By *Harmen G. van Lessen, Mario Stefanini and Francis E. Smith.* Joseph Stanton Memorial Laboratories, Saint Elizabeth's Hospital, and Department of Medicine, Tufts University Medical School, Boston.

Four cases of severe erythrocytosis associated with renal cell carcinoma have been studied during the past 3 years. White cell and platelet count appeared unaffected. Common finding was varicocele and, at surgery, invasion of the renal vein by tumor tissue. No erythrocytosis was observed in other renal cell carcinomas without thrombosis of a renal vein.

To investigate the role of venous stasis within the kidney in the development of erythrocytosis, partial ligation of the left renal vein was performed in dogs, diameter of the vessel being reduced to approximately 1/5 of the original. All animals developed persistent albuminuria and microscopic hematuria. The majority responded with an appreciable increase in hematocrit, total blood and red cell volumes determined by the dye technic. The behavior of white cells and platelets was erratic and not consistently elevated. Additional ligation of the left spermatic vein did not influence results. Maximum response was observed between the 8th and 12th week after operation. In a typical experiment blood volume, red cell volume and hematocrit rose in 10 weeks from 1156 ml., 578 ml. and 50% to 1760 ml., 1039 ml. and 58% respectively. Values later declined, returning to normal within 16 to 20 weeks. At autopsy all treated kidneys were enlarged and had developed extensive venous bypassing anatomoses, exhibiting increased venous pressure. Microscopic studies revealed venous congestion of glomeruli, cloudy swelling of the tubular epithelial cells. *Conclusions:* Erythrocytosis follows partial occlusion of a renal vein in dogs. It is transitory, probably because of the development of adequate collateral circulation. The engorged kidney may produce an erythropoietic factor.

### Studies of Plasma Erythropoietic Factor in Man

By *Paul R. McCurdy, Emil Miskovsky, Theodore Laughlin and C. Fred McCuiston.* Georgetown Medical Division of D.C. General Hospital, and Department of Medicine, Georgetown University Medical School, Washington, D.C. (Aided by a grant from the Washington Heart Association.)

Since most studies concerning the generally accepted plasma erythropoiesis stimulating factor have used animals as test indicators for human or animal material, the present study was undertaken to demonstrate that such a factor was active in humans.

ACD plasma obtained from suitable donors was freshly frozen and stored until use. The Miller disc was used for reticulocyte counts. Other technics were those in standard use.

A normal human volunteer (72 Kg.) was given 200 ml. of plasma from secondary polycythemia patients (Hct. over 55%) daily for 5 days. His hemoglobin and hematocrit did not change. His reticulocytes average 1.0% (0.6–1.4%) during the control period and reached a peak of 1.9% on the 5th experimental day. A 2nd volunteer (73 Kg.) was given 500 ml. of plasma from patients with sickle cell anemia daily for 5 days. Aside from hemodilution, the only significant change was in reticulocytes which averaged 1.2% (1.0–1.3%) control and rose to 2.2% on the 7th day and was above 1.7% on 4 other occasions commencing with the 3rd day.

A 6-year-old Negro child (22 Kg.) with Blackfan-Diamond congenital aplastic anemia was given 200 ml. daily for 5 days on one occasion of sickle cell plasma and on another of anemic-by-bleeding plasma. No evidence for stimulation of erythropoiesis was observed.

Sickle cell plasma and probably secondary polycythemic plasma contain a factor capable of stimulating erythropoiesis in normal humans. Sickle cell plasma and anemic-by-bleeding plasma did not stimulate erythropoiesis in a child with pure red cell aplasia.

### The Utilization of Erythropoietin

By *Frederick Stohlman, Jr.* National Institutes of Health, Bethesda.

The level of erythropoietin in the plasma and urine of some patients with refractory anemia was found to be substantially greater than in patients with similar hemoglobin levels as the result of blood loss or hemolytic anemia. This

apparent discrepancy could result from a greater utilization of erythropoietin by the hyperplastic than by the normal or hypoplastic marrow. To explore this possibility the rate of appearance and disappearance of erythropoietin in intact rats was compared with that of rats with radiation (400 r) induced hypoplasia.

The release of erythropoietin was evoked by exposure at a simulated altitude of 23,000 feet or the removal of a quantity of blood equivalent to 2% of the body weight. Plasma erythropoietin content was assayed in fasted rats using $Fe^{59}$ incorporation as a measure of red cell production. Erythropoietin was demonstrated in the normal animal 2 hrs. after exposure to simulated altitude and reached a peak within 12–24 hrs. Thereafter in both bled and altitude-exposed animals the level of erythropoietin fell, so that at 48 hrs. the activity was substantially diminished and after 90 hrs. of continuous exposure could no longer be consistently demonstrated. In contrast, in rats in which red cell production was suppressed by ionizing radiations (400 r), the level of erythropoietin following 48 hrs. of exposure at an altitude of 23,000 feet was significantly greater than in the nonirradiated group. Moreover, following an exposure of 90 hrs., significant erythropoietic activity was still present in the plasma of the irradiated group.

These studies support the concept that erythropoietin not only stimulates red cell production but is utilized in the process. The plasma level of erythropoietin, then, reflects the balance between production and utilization.

### Clinical Experiences with Erythropoietin

By *A. Leonard Luhby, Jack M. Cooperman, Julia M. Herrero, Albert S. Gordon, Sam J. Piliero and Paul T. Medici.* Departments of Pediatrics and Anatomy, New York Medical College; Departments of Biology, Graduate Schools, New York University and St. John's University. (Aided by grants from the Playtex Park Research Institute; Division of Cancer Control and Research, New York City Department of Health; and the Intercounty Blood Banks, Inc.)

Studies were undertaken to determine whether erythropoietin preparations found to be active in laboratory animals would stimulate erythropoiesis in certain anemias in humans.

Three different types of erythropoietin preparations were used in 3 different types of anemia. One was plasma from severe Cooley's anemia subjects of the same blood type as the recipient. The 2nd was urine from an anemic acute leukemia, Hb. 6.5 Gm.%. The 3rd was an acidified, boiled filtrate of plasma from dogs made anemic with phenylhydrazine. All preparations were erythropoietically active in intact rat assays.

The clinical material included one patient with chronic hypoplastic anemia. His marrow contained 0.5 to 1.0% erythroid cells, but was otherwise normal. Erythropoietic Cooley's plasma, 735 ml., was given i.v. in 9 days. No reticulocytosis occurred and his Hb. fell from 8.0 to 3.5 Gm.% during a daily 3-week observation. The marrow remained unchanged. The 2nd patient, a 2½-month-old infant with anemia of prematurity, received 600 ml. of dog plasma filtrate orally over 20 days. Her twin received a placebo. Daily counts revealed an increase of Hb. from 8.5 to 9.6 Gm.% in the patient, compared to 8.5 to 9.0 Gm.% in the control. There was a slight reticulocytosis in both infants, but no change in RBC in either. The third patient, a 3-month infant with accentuated "physiological" anemia following mild erythroblastosis fetalis, received 185 ml. of leukemic urine orally over 5 days. There was no change in daily RBC and reticulocyte counts for one month; the Hb. fell from 8.7 to 7.6 Gm.%

There were no significant erythropoietic responses. The chronic hypoplastic anemia may have been incapable of response; none occurred during subsequent steroid therapy. The infants may not have responded because "erythropoietin" may be inactive orally.

### Painful Erythropoietic Crises Produced by "Pharmacologic" Doses of Folic Acid

By *James H. Jandl and Mortimer S. Greenberg.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston.

Many examples have been reported of "aplastic crises" complicating chronic hemolytic anemias. Infections frequently precipitate these crises and occasionally maturation arrest of the marrow has been noted. The following observations on a woman with Cooley's anemia indicate that in some instances a specific, correctable metabolic imbalance may underly episodes of marrow failure.

Within 4 years this patient developed 3 episodes of progressive anemia and reticulocytopenia associated with hyperplasia of primitive erythroblasts and high levels of fecal urobilino-

gen. Numerous substances involved in normal erythropoiesis were administered without effect, including daily injections of 400 μg. of folic acid, an amount sufficient to correct simple deficiency. In all instances, however, oral administration of 5 or 10 mg. of folic acid exerted a prompt erythropoietic effect. Moreover, it precipitated severe generalized bone pain, particularly in the pelvis, spine, femora and rib cage. These pains began 12 hours after therapy and lasted 36 hours. They were associated with marked normoblastic hyperplasia and a fall in serum levels of iron and vitamin $B_{12}$. Shortly thereafter nucleated red cells appeared in the peripheral blood, followed by striking reticulocyte responses. The hemoglobin values then rapidly returned to their usual levels. The painful crises were presumably caused by acute erythroid proliferation and increased intramedullary pressure.

Dietary deficiency of folic acid did not exist and impaired intestinal absorption was not demonstrable. The patient's urinary levels of folic acid were normal before therapy and she excreted orally-administered folic acid promptly. Her urine did not contain formiminoglutamic acid. It is concluded that in this hemolytic disorder, "pharmacologic" amounts of folic acid, or its metabolic derivatives, were necessary for sustained erythropoiesis, and that relative insufficiency of specific metabolites may precipitate some forms of aplastic crisis.

### The Mechanism of Decreased Erythropoiesis in Experimental Polycythemia

By *Clifford W. Gurney and Chao Pan.* Department of Medicine, University of Chicago, and Argonne Cancer Research Hospital, USAEC.

Employing bioassay procedures, numerous investigators have demonstrated an erythropoietic-stimulating hormone (erythropoietin) in extracts of plasma from anemic animals and human beings. Small amounts of erythropoietin are present in plasma from normal donors. These findings suggest that erythropoiesis is regulated by plasma erythropoietin concentrations and that a reciprocal relationship exists between the plasma erythropoietin concentration and the hemoglobin level. We have studied the relationship between erythropoietin and the depression of erythropoiesis occurring in rats made polycythemic by transfusion.

Following a single injection of homologous erythrocytes, erythropoiesis, as measured by the reticulocyte count and the rate at which a tracer dose of $Fe^{59}$ is incorporated into erythrocytes, decreased steadily. The degree of this suppression was related to the degree of polycythemia produced until erythropoiesis was almost completely inhibited following transfusion of packed cells in the amount of 4% of the recipient's body weight. After 2 daily injections of anemic rat plasma or the filtrate of heat-denatured anemic human plasma, erythropoiesis in polycythemic rats, as measured by the 16-hr. erythrocyte incorporation of $Fe^{59}$, was increased 6-fold when compared with the incorporation following normal plasma or plasma extract. After 9 injections of cobaltous chloride, the reticulocyte count of polycythemic rats rose steadily to an average of 2.6% (saline control 0.3%), and the 16-hr. $Fe^{59}$ incorporation was 32% (saline control 5%).

We conclude that the suppression of erythropoiesis in the polycythemic rat can be reversed by plasma or plasma extracts containing increased amounts of erythropoietin and by the administration of cobalt, a substance recently shown by Goldwasser, Jacobson, Fried and Plzak to increase the erythropoietin titer in normal animals. These experiments support the concept of the hormonal regulation of erythropoiesis by suggesting that decreased erythropoiesis in the polycythemic rat is a consequence of decreased erythropoietin concentrations.

### Differential Extraction of Nucleoprotein from Human Leukocytes

By *Charles C. Sprague, Richard T. Green and Ana E. Carrera.* Department of Medicine, Tulane University School of Medicine, New Orleans. (Aided by a grant from the United States Public Health Service.)

Laves and Thoma have reported that with the incubation of methanol-fixed peripheral blood or bone marrow films in urine dialyzed against tap water, the nuclear chromatin of the neutrophilic granulocytes is lysed, whereas the chromatin of all other leukocytes remains unaltered. They attribute this to a "ribonuclease complex" in urine, and as a result state that the cells of the myelocytic series contain nuclear nucleotides which are predominantly of a ribose nature, whereas lymphatic and monocytic cells contain desoxyribonucleotide almost exclusively.

The authors have confirmed that buffered fresh, undialyzed urine or urine dialyzed against tap water "lyses" the nucleoprotein of the neutrophilic leukocytes without affecting that of the

lymphocytes or monocytes. However, pure crystalline ribonuclease failed to produce these changes, as did urine dialyzed against distilled water. The action of urine was closely simulated by incubating the slides in .07 M NaCl and .05 M phosphate buffer solutions. The reaction was not potentiated by the addition of pure crystalline ribonuclease.

The use of salt solutions of varying concentration for the extraction of nucleoprotein is a standard procedure, and it is our impression that the dissolution of the nucleus of the granulocyte when incubated in urine is due largely to the fact that urine is basically a salt solution. The possibility of enzyme activity has not been excluded, however, and it is possible that proteolytic enzymes present in urine, particularly proteases, may play a role. Trypsin and chymotrypsin produce alterations in the nucleus but do not have any significant differential effect. The differential effect observed could be explained on the basis of a difference in binding between the nucleic acid and the basic protein of granulocytes, as compared to the mononuclear cells or possibly on differences in cell permeability.

### Studies of Erythropoiesis in Acute Leukemia

By *David G. Nathan and Nathaniel I. Berlin.* Metabolism Service, General Medicine Branch, National Cancer Institute, Bethesda.

Diminished red cell life span is believed to be the major cause of anemia in chronic leukemia. The purpose of this research is to define some of the disturbances of erythropoiesis and red cell survival during the course of acute leukemia.

Eight studies were performed in 7 adult patients with acute leukemia. The studies included measurement of plasma and red cell iron turnover with $Fe^{59}$, lifespan of the erythrocyte with $Cr^{51}$ and glycine-2-$C^{14}$, and kinetics of $Cr^{51}$ and $Fe^{59}$ in spleen, liver and bone marrow. The Coombs' test, measurement of fecal urobilinogen, and hemoglobin electrophoresis were performed in addition to the peripheral blood counts and bone marrow examination.

The results indicated deficient erythropoiesis in 4 patients and absolute bone marrow failure to produce red cells in one. Three of these patients also demonstrated a short red cell lifespan with sequestration of red cells in the spleen, liver or both. One patient with increased bone marrow function presented suggestive evidence for the production of two populations of red cells both with short survivals and with sequestration in the spleen and liver. The plasma iron was found to be cleared by the liver rather than the marrow in the patients with deficient erythropoiesis. The bone marrow examinations did not reliably predict the activity of erythropoiesis. This was expected in view of the well known sampling error inherent in aspiration of pathologic marrow.

Acute leukemia may produce shortening of the red cell lifespan, but acute leukemia appears to differ from chronic leukemia in that a significant number of patients with acute leukemia demonstrate a marked deficiency in erythropoiesis.

### Leukotoxicity of Pathologic Sera and Certain Drugs

By *Stuart C. Finch and Katherine D. Detre.* Department of Internal Medicine, Yale University School of Medicine, New Haven.

The purpose of this study was to evaluate the leukotoxic effects of certain pathologic sera and drugs as measured by depression of human leukocyte starch granule phagocytosis. This method of evaluating leukocyte injury is simple, reliable and sensitive. It also has the advantage of being able to differentiate intra- from extracellular factors responsible for the development of leukocyte damage. Variations in degrees of normal starch granule phagocytosis due to differences in serum complement and starch antibody concentrations are eliminated by using starch granules sensitized with serum known to contain normal amounts of these substances. The technic involves incubating normal leukocytes with test serum or drug for 1 hr. and then with sensitized starch for 30 minutes at 37 C. If leukocyte injury has been sustained, less than 70% of the polymorphonuclear leukocytes will contain starch.

Results of approximately 175 tests indicate significant depression of phagocytosis with 18 of 20 L.E. sera, 4 of 6 multiple transfusion sera, and 10 heterologous antinuclear and antileukocyte sera. Physiologic amounts of chloramphenicol, P.A.S. and versene also produced positive results. No depression was found with 20 isologous human sera, normal rabbit serum, and serum from 17 leukopenic flu patients. Negative results were obtained with leukocytes from patients with either active tuberculosis or positive tuberculin skin tests when incubated with P.P.D.

These studies demonstrate a specific pathophysiologic effect of lupus factor and certain leukotoxic substances on human leukocytes. The

technic appears to be useful in testing for antileukocyte factors.

### Cryofibrinogenemia Associated with Thrombophlebitis Migrans in a Patient with Acute Leukemia

By *Emil J. Freireich and Carmella Macri.* General Medicine Branch, National Cancer Institute, Bethesda.

The etiology of thrombophlebitis migrans associated with malignant disease is unknown. The properties of a cold precipitate found in the plasma of a patient with this syndrome were studied.

The patient, a 60-year-old female with acute myeloblastic leukemia, on admission had 300,000 WBC/mm.$^3$, active thrombophlebitis and a 1-month history of thrombophlebitis migrans. At this time, cryofibrinogen was found in her plasma. 6-mercaptopurine therapy resulted in partial remission and cryofibrinogen disappeared. One month later relapse occurred and cryofibrinogen reappeared. Methotrexate therapy induced leukopenia and thrombopenia with disappearance of cryofibrinogen.

Precipitation occurred in plasma at 4 C. within 2 hours, with a maximum at 18 hours. No precipitation occurred in serum at 4 C. or in plasma at 37 C. The precipitate dissolved at 37 C. and clotted with addition of thrombin, identifying it as "cryofibrinogen." Citrate, oxalate and EDTA anticoagulants gave similar quantities of precipitate, while heparin gave 2 to 5 times this quantity. The washed cold precipitate was dissolved and studied in the ultracentrifuge at 24 C. Four major components were found with sedimentation constants of 11.4, 9.6, 8.0 and 6.8, all X $10^{-13}$ in 0.3 M KCl. After reprecipitation in the cold, 2 major components, S = 12.2 and 10.2 remained. Addition of thrombin clotted 40% of the protein. The remaining soluble protein showed a single major component, S = 12.4. It was soluble at 4 C. and would not clot with thrombin. This protein precipitated with the fibrinogen during 4 consecutive reprecipitations at 4 C. The clottable portion of the cold precipitate was 33–52% of the total, and showed poor solubility at low salt concentrations.

The cryofibrinogen described consisted of (1) polymerized fibrinogen and (2) an unidentified cold soluble protein. Both components were required for the development of a precipitate. The presence of the cryofibrinogenemia was associated with thrombophlebitis migrans and correlated with the activity of the acute leukemia.

### Bone Marrow Maturation Disturbance in Early Leukemia

By *Erwin O. Hirsch and Thomas S. Micolonghi.* Medical Service and Institute of Pathology, Rhode Island Hospital, Providence.

In ten cases of early leukemia initial findings were anemia, leukopenia or thrombocytopenia alone or in combination. There was minimal or no organ enlargement and there were no immature cells in the peripheral blood. Evidence of hemolytic phenomena was lacking. The bone marrow showed only very slight increase in immature cells, so much so that the diagnosis of leukemia was often in doubt when patients were first examined. Evidence of a maturation arrest, such as myeloid arrest and macronormoblasts, was present in the bone marrow. Anisocytosis and poikilocytosis were common in the peripheral blood. Eventual progression to the characteristic leukemic bone marrow replacement occurred after 6 months to 2 years.

It is concluded that in some patients with leukemia, there is an early maturation disturbance in the bone marrow which is responsible for varying cytopenias before there is evidence of marked bone marrow invasion. This maturation disturbance might be explained by a greater avidity for metabolites by the leukemic cells than by normal cells. Alternately, these observations raise the question of a common cause for both the leukemia and the associated maturation disturbance of the nonleukemic bone marrow.

### Methylated Mylerans (CB2348) and (CB2348K) in the Treatment of Leukemia and Neoplastic Diseases in Man

By *Keith H. Kelly, Howard R. Bierman, Fauno L. Cordes and Geoffrey Timmis.* Department of Internal Medicine, Section of Oncology, City of Hope Medical Center, Duarte, California, and Chester Beatty Research Institute, London, England.

Methylation and isomerization of the sulfonoxy butanes alters the solubility and permeability characteristics of Myleran which confers an ability to interfere promptly and irreversibly with nuclear and cytoplasmic regenerative mechanisms of immature leukocytes and human ascites tumor cells.

Eleven patients with chronic granulocytic leukemia received 24 courses of CB2348 or its isomer CB2348K intravenously in doses of 0.1 to 0.8 mg./Kg. body weight. A prompt decrease in

absolute granulocyte number followed in each instance in direct relationship to the dosage. The immature granulocytes appeared to be preferentially although not exclusively affected. Clinical improvement accompanied the reduction in hypercellularity of the marrow and blood. This response persisted for 2 to 12 weeks following a single injection and could be repeated. In 3 patients no longer responsive to oral Myleran or x-ray therapy, CB2348 induced clinical and hematologic improvement for 1 to 2 months following a single injection.

Seventeen courses of CB2348 or CB2348K were administered to 12 patients with wide spread nonhematologic neoplastic diseases. Intraperitoneal administration of 0.5 to 1.2 mg./Kg. body weight in 5 patients with abdominal ovarian carcinomatosis caused regression of ascites formation and profound cytologic abnormalities and reduction in tumor cell number.

Dimethyl Myleran (CB2348) or its isomer (CB2348K) are effective soluble alkylating agents in the treatment of chronic granulocytic leukemia, inducing a prompt predictable response following parenteral administration.

### A Critical Appraisal of Steroid Therapy in Acute Leukemias

By *Edward Shanbrom*. Department of Medicine, Section of Hematology, City of Hope Medical Center, Duarte, California.

Reports have appeared suggesting that massive doses of steroids are more beneficial than conventional doses in the treatment of acute leukemia. An attempt at critical evaluation of this therapy was made by utilizing the "double blind" method of drug administration. Patients received either 50 or 500 mg. of prednisolone daily for 10 days. CBC's were performed at regular intervals and bone marrow specimens were obtained before and after the 10-day period. Patients unresponsive to 50 mg. doses were treated with 500 mg. for an additional 10 days. Of 50 patients studied, 22 had granulocytic, 21 lymphocytic, 6 monocytic and 1 plasma cell leukemia.

Although most patients exhibited initial subjective improvement, there was no hematologic improvement in any cases of granulocytic, monocytic or plasma cell leukemia regardless of the dosage. Nine patients appeared to be made worse. All of the patients with lymphocytic leukemia showed some hematologic benefit and 12 went into complete remission with this form of therapy alone. Hematologic improvement appeared more rapidly with larger doses of prednisolone in "steroid sensitive" patients, but there was little evidence that smaller doses would not have brought about similar results though less quickly. In 3 patients, 500 mg. doses appeared more efficacious than conventional doses. In 6 patients unresponsive to 500 mg., as much as 6000 mg. of prednisolone daily failed to induce hematologic improvement.

There were 11 cases of fulminant septicemia in 38 patients receiving 500 mg. doses; 7 patients developed severe perirectal abscesses.

It would appear from this study that prednisolone is not an effective antileukemic agent in acute granulocytic or monocytic leukemia; there is evidence that it may accelerate the disease process in some patients. In contrast, steroids are effective in acute lymphocytic leukemia and may predictably induce hematologic improvement. Massive doses may induce remissions more rapidly than smaller doses, but complications are too serious to recommend their use routinely.

### Nutrient and Energy Metabolism in Patients with Myeloproliferative Disease

By *Donald M. Watkin*. Metabolism Service, National Cancer Institute, Bethesda.

Five patients with chronic myelocytic leukemia and one with myeloid metaplasia were studied by the metabolic balance and indirect open-circuit calorimetry technics to measure changes in body composition and in energy metabolism induced by treatment of the myeloproliferative disease, in these cases with Myleran or Colcemide.

During control periods, the patients generally were in balance with respect to N, K, P, Ca, Na and Cl. Their blood levels and urinary excretions of uric acid exceeded those of patients without myeloproliferative disease on comparable diets. Their total energy expenditures were high, their BMR's normal or high and their basal R.Q.'s normal or low. In some, N/P balance ratios suggested formation of neoplastic tissue.

Treatment was associated with falls in WBC's, rises in blood and urinary uric acid, negative imbalances of nitrogen and phosphorus, gradual reductions in total energy expenditures and BMR's and rises in the basal R.Q.'s. The N/P balance ratios suggested the lysis of neoplastic tissue.

Remissions generally were characterized by nutrient balance, blood and urine uric acid values below control levels, normal total energy ex-

penditures, normal or low BMR's and normal or elevated R.Q.'s.

The N/P balance ratios were within a normal range.

Clinical remissions with Colcemide have been noted in certain patients who entered relapse on Myleran therapy.

### Chromatographic Studies on Platelet Phospholipids

By *Aaron J. Marcus and Theodore H. Spaet.* Department of Hematology, Division of Laboratories, Montefiore Hospital, New York. (Aided by a grant from the U. S. Public Health Service.)

Phospholipid preparations from a number of sources can replace platelets in blood coagulation tests. This is especially true in thromboplastin generation. The purpose of this study was to separate and identify the components of crude phospholipid extracts of human platelets by means of paper and column chromatography.

Chloroform extracts of acetone-dried blood platelets were chromatographed on silicic acid impregnated paper using 3 different solvent systems. With phenol, ether, acetone and water, 2 fractions were obtained. The first, with an Rf value of 0.93 was strongly positive when stained with ninhydrin. Samples of pure ethanolamine phosphatide behaved the same way. The 2nd component had a Rf value of 0.7 and was strongly positive when stained for choline. Pure lecithin and sphingomyelin ran with this spot. With a solvent system composed of 20% methanol in chloroform, the 2nd spot could be fractionated into 2 components with Rf values of 0.3 and 0.4 respectively. These could be identified as sphingomyelin and lecithin. Using diisobutyl ketone: acetic acid: water (40:25:5, v/v) 5 fractions were tentatively identified. The leading spot, with an Rf of 0.89 was ethanolamine phosphatide. This was followed by serine phosphatide, lecithin, sphingomyelin and inositol phosphatide. These chromatograms were stained with Rhodamine 6G and viewed under ultra violet light.

Using silicic acid, crude platelet phosphatides were subjected to column chromatography. The eluates were rechromatographed on paper, and separation of the "cephalins" from the "lecithins" was obtained.

An attempt was made to test the fractions for coagulant and anticoagulant activity. Thus far, these could not be adequately suspended for use as platelet substitute in the thromboplastin generation test.

### Preservation Studies of Dog Platelets

By *Phin Cohen, James C. Pringle and Frank H. Gardner.* Curtis Hematology Laboratory, Peter Bent Brigham Hospital, and Department of Medicine, Harvard Medical School, Boston. (Aided by a grant from the Department of the Army, Office of the Surgeon General.)

Platelet survival time by the $Na_2Cr^{51}O_4$ technic has been adapted to dogs and normally is 7 to 9 days. The survival times are identical in the dog using $EDTANa_2$ or A.C.D. as the anticoagulants. This contrasts with human studies in which an abbreviated platelet lifespan is observed when A.C.D. is used as the anticoagulant.

Because of the ease in handling dog platelets in A.C.D. anticoagulant, experiments were designed to determine the effects of storing platelets in whole blood, platelet-rich plasma and platelet concentrates. Attempts have been made to correlate the survival of stored platelets with their clot retraction function. Clot retraction has been semiquantitated and measured by using various concentrations of platelet suspensions ranging from 150,000 to 5,000 in order to assess the approximate percentage of viable platelets remaining after storage.

Platelets can be stored in whole blood and platelet-rich plasma for periods up to 9 hours at 4 C. with no loss of clot retraction or change in the duration of the survival curve of $Cr^{51}$-tagged platelets after infusion in the dog. Storage periods from 9 to 12 hours result in a diphasic curve, reflecting 2 populations of tagged platelets in vivo; one with abrupt destruction, and the other with normal survival. Intervals beyond 12 hours result in abrupt destruction by the infused platelets, but clot retraction is retained. This apparent discrepancy between parameters of viability may be explained by the fact that clot retraction studies have been performed with nonmanipulated platelet-rich plasma, whereas platelet transfusions are manipulated by differential centrifugation for $Cr^{51}$-tagging before infusion.

Platelet concentrates can be stored for periods up to 6 hours with no change in the survival pattern of tagged platelets or in clot retraction of the resuspended platelets.

### Investigations of the Mechanisms of Thrombocytopenia

By *Frank H. Gardner, Knut A. Aas, Phin Cohen and James C. Pringle.* Curtis Hematology Laboratory, Peter Bent Brigham Hospital, and Department of Medicine, Harvard Medical School,

Boston. (Aided by a grant from the Department of the Army, Office of the Surgeon General.)

Previous studies have demonstrated that human blood platelets tagged with $Na_2Cr^{51}O_4$ have a lifespan of 8 to 11 days when infused into normal recipients. Observations have been made on 51 patients with a variety of hematologic disorders to evaluate platelet destruction. Patients with autoimmune thrombocytopenia purpura have had a shortened lifespan of tagged platelets. A reversion to a normal pattern may be observed during corticoid treatment or following splenectomy. Several patients with chronic idiopathic thrombocytopenia have demonstrated a normal lifespan of isologous and autologous tagged platelets associated with a normal cytologic pattern of megakaryocytes. In such instances the lifespan of tagged platelets has been useful in excluding autoimmune disorders.

The thrombocytopenia related to congestive splenomegaly and portal hypertension has been associated with a normal survival period of tagged platelets. These observations suggest that the platelet depression in this disorder is caused by bone marrow inhibition of platelet formation rather than peripheral destruction due to splenic sequestration.

Evaluation of splenic, liver and pulmonary sequestration of tagged platelets by body-surface counting with a scintillation probe has not demonstrated localization of radioactivity to indicate sites of platelet destruction in any hematologic disorder studied.

This $Cr^{51}$ technic has allowed determination of lifespan of autogenous tagged platelets when peripheral blood platelet levels are as low as 80,000 $mm^3$. Patients who have received multiple tranfusions will in some instances have decreased tagged platelet lifespan reflecting previous isoimmunization from ABO compatible transfusions. It is anticipated that information regarding the lifespan of platelets may be helpful in classification and therapy of thrombocytopenic disorders.

### Observations on Hemorrhagic Disorders Associated with Platelet Dysfunction

By *Shirley A. Johnson, Raymond W. Monto and John W. Rebuck.* Department of Laboratories and Division of Hematology, Henry Ford Hospital, Detroit.

Usually platelet diseases are considered in terms of platelet numbers; in clinical medicine it is observed that individuals with normal or increased numbers of platelets manifest bleeding of a type which suggests platelet dysfunction. These include syndromes classified as pseudohemophilia, agnogenic myeloid metaplasia, polycythemia vera, complications following dextran administration, uremia and thrombocytopathy.

These catagories of patients uniformly exhibited abnormal prothrombin consumption. Other coagulation components in this group appeared to be within normal range. Platelets of patients in the group under consideration were isolated and assayed for known platelet activities. In addition platelet abnormalities were observed with the electron microscope. Platelet granules were obtained and then morphology and activity related.

Insofar as these quantitative evaluations can be understood, all instances of poor prothrombin consumption were related to reduced platelet factor 3 activity. In the case of thrombocytopathy A, the defect appeared to be in relation to the liberation of platelet factor 3 rather than content. However, in uremic states the platelet factor 3 activity does not appear to be present at least in its active, available form. The platelet fibrinogen content of platelets of one patient was markedly decreased, associated with normal plasma fibrinogen. These findings suggest a 3rd type of platelet functional disturbance, thrombocytopathy C.

### The Thromboplastin-Heparin Time of Human Plasma

By *Sean M. Lavelle and George P. Thompson.* Department of Pathology, Galway Medical School, Ireland.

The clotting time of heparinized plasma on the addition of tissue extract was studied to see if results correlated with age, sex or arteriosclerotic disease.

Three ml. of whole blood were added to 9 units of heparin in a test tube and mixed well. Duplicate tubes were taken on each subject. The plasma was separated, and 0.2 ml. clotted with 0.1 ml. of rabbit brain suspension at 37 C. The hematocrit was determined, and the results of the test were expressed in clotting time in seconds per unit of heparin per ml. of plasma.

Twenty medical students, of whom 3 were women, were used as controls. Patients taken at random from the medical wards were examined, 27 females and 37 males. Half of them were less than 50 years old, some from each decade.

Mean clotting time of the control women was insignificantly less than that of the men.

Among the patients, the females showed a slightly longer clotting time than the males in each decade save the 4th. The mean clotting time of both sexes decreased with age from 13.1 seconds in the 2nd decade to 11.1 seconds in the 8th.

The mean clotting time for 8 cases of cerebral arteriosclerosis was 10.6 seconds. For 8 cases of cardiac arteriosclerosis, it was 11.1. One hypertensive had a short time, 4 did not. Three diabetics did not have a shortened time.

The results suggest that there is a slightly increased blood coagulability occurring with age that is slightly more marked in men and is associated in a way with arteriosclerosis.

**Mechanism of a Circulating Anticoagulant Producing a Hemophilia-like Disorder in a Female**

By *Jacques Gauthier, Herbert S. Sise and Robert Becker.* Anticoagulant Laboratory, Boston City Hospital, and Department of Medicine, Tufts University School of Medicine. (Aided by a grant from USPHS.)

There have been several cases reported of a hemophilia-like bleeding diathesis, often in females, usually beginning a few months after a normal pregnancy, and due to a circulating anticoagulant directed against antihemophilic globulin. Herein is described such a case with observations on the variability of the anticoagulant and the level of AHG. The anticoagulant was demonstrated by calcium clotting times, thromboplastin generation test and partial thromboplastin times on mixtures of normal and patient's plasmas. Moreover, there could be shown a specific antagonism to AHG by destruction of the corrective effect of normal plasma or Fraction I on hemophilic plasma. AHG levels were assayed by a one-stage test. Followed over the course of 3 months, including a 2-week period of high dosage of steroids, the anticoagulant and AHG showed wide fluctuations. Three patterns were observed: (1) a high level of anticoagulant and low level of AHG; (2) absence of detectable anticoagulant and low AHG (a picture simulating true hemophilia); (3) absence of anticoagulant and normal AHG (one observation only). At no time were significant AHG levels detectable in the presence of the anticoagulant. These circumstances support the hypothesis that the inhibitor and AHG mutually neutralize each other. An improvement following steroids could not be positively attributed to the medication because of failure to relapse after withdrawal and the spontaneous changes during and after steroids.

**Experimental Hemarthrosis: Clearance of the Radioactivity of Chromium$^{51}$-labeled Erythrocytes Injected into the Knee Joint of Rabbit and Man**

By *Gerald P. Rodnan.* Department of Medicine, University of Pittsburgh, Pennsylvania.

Hemarthrosis, affecting most commonly the knees, ankles and elbows, constitutes the most common major hemorrhagic complication in adult hemophilia. Repeated bleeding is frequently associated with permanent deformity and disability of one or more of the affected joints. To learn more concerning the disposition of such joint hemorrhage, 1 cc. of homologous erythrocytes labeled in vitro with $Na_2Cr^{51}O_4$ was injected into the knee cavity of rabbits, and the area of the joint monitored by means of an external scintillation probe. Under "natural" cage conditions there was a rapid decline in radioactivity so that 5 days after injection approximately 25% of the original chromium$^{51}$ activity remained within the knee joint segment. Thereafter, the fall in count was much slower, appreciable quantities of radioactive chromium (presumably bound to globin) remaining in situ for many weeks. Application of a cast to the injected extremity appeared to delay transiently the disappearance of $Cr^{51}$ activity from the knee. Intrasynovial administration of neither hyaluronidase (150 U.S.P. units) nor of hydrocortisone (25 mg.) appeared to influence the rate of extent of removal of injected radioactivity.

To study the clearance of joint hemorrhage in man, 10 cc. of autogenous chromium$^{51}$-labeled erythrocytes were injected into one or both knee joints of 4 adult hemophilic patients who were receiving plasma for the control of (other) hemorrhage. These patients were at bed rest. In each case there was a gradual decline in radioactivity, so that by the 10th day after injection 35–55% of the original count remained in situ. After this, there was little if any change in the chromium$^{51}$ activity in the area of the joint. These findings suggest that following hemarthrosis appreciable quantities of globin remain within the area of the joint indefinitely.

**Acquired Plasma Thromboplastic Component Deficiency**

By *A. A. Cintrón-Rivera, M. V. de Torregrosa and R. S. Díaz-Rivera.* Departments of Medicine

and Pathology, School of Medicine, and San Juan City Hospital, San Juan, Puerto Rico.

As a rule, plasma thromboplastic component deficiency, or hemophilia B, is a sex-linked recessive hereditary disorder which is seen practically always in males. It is characterized by severe bleeding, remissions and exacerbations, with the defect persisting throughout life. The defect is corrected by the administration of blood, serum or plasma in any form, but it is not corrected by the use of corticoids.

The case under consideration presented some striking differences to the usual picture of P.T.C. deficiency. It consisted of an acquired and apparently self-limiting form of the disease, which appeared in a 53-year-old mulatto woman, complicating a severe reaction to penicillin. There was no family history of bleeding tendencies. The patient had had 3 normal deliveries without complications. No coagulation defects existed in the family.

From the time of the onset in 1954, she had 3 prolonged hospitalizations at the San Juan City Hospital because of serious bleeding manifestations. Extensive studies were performed and the coagulation defect was characterized as a P.T.C. deficiency or hemophilia B. The coagulation time and the prothrombin consumption tests were abnormal. The thromboplastin generation test confirmed the diagnosis of a P.T.C. deficiency. The deficiency *was not* corrected by barium sulfate absorbed plasma or by P.T.C. deficient plasma, but it *was* corrected by fresh and old plasma, blood and serum, and by hemophilic (hemophilia A) blood. The bleeding, prothrombin times, platelet counts, clot retraction and fibrinogen levels were normal.

Repeated and adequate responses to the administration of corticoids, as well as to plasma, serum or blood were obtained. The defect was present for 2½ years, but, 3 years after the onset of her disease, she seems to have recovered completely as shown by clinical and laboratory tests.

### Effects of Calcium-binding Anticoagulants on the Therapeutic Effectiveness of Plasma in AHG and PTC Deficiency

By *N. Raphael Shulman, Thomas C. Bithell and Joel H. Feigon.* National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda.

Several investigators have reported that anticoagulants which bind Ca++ strongly—particularly EDTA—may decrease the accelerator activity of plasma and the PTC activity of serum. No data, however, are available on the effects of EDTA on AHG, on PTC in plasma, or on the effectiveness of EDTA-plasma in the correction of hemophiliac patients. The present report summarizes studies on the comparative effects of EDTA-plasma, heparin-plasma, and ACD-plasma administered to 2 severe AHG-deficient and 2 severe PTC-deficient patients.

The effects of a standard dose of 0.45 cc. plasma/Kg. body weight on the glass-clotting time, two-stage prothrombin consumption and recalcification time, were determined. In each experiment, aliquots of fresh plasma in standard ACD solution, 1.5% EDTA, and 0.6 μg. heparin/ml. (all 1 volume to 9 volumes of blood) were obtained from a single donor.

In AHG deficiency, ACD-plasma was as effective as heparin-plasma, indicating that the Ca++ binding effect of citrate in the concentrations used did not inactivate AHG. EDTA-plasma, in sharp contrast, had only the effect of approximately 1/3 the standard dose of ACD-plasma. In vitro studies demonstrated a failure of EDTA-plasma to correct the abnormal thromboplastin generation test and recalcification time of AHG deficient plasma in the presence of any concentration of calcium.

In PTC deficiency, however, EDTA-plasma had a maximal effect as great as that of ACD-plasma, but required 8 to 10 hours to attain this effect, compared to ½ to 1 hour with ACD-plasma. The duration of the effect was longer than with ACD-plasma.

The data suggest (1) that AHG is altered by EDTA, possibly by removal of Ca++ from a complex or molecule. Preliminary work will not allow conclusions as to the reversibility of this inactivation. (2) PTC is so altered that it gradually attains maximal activity in vivo. Recalcification of a complex or molecule could account for these observations.

### The Quantitative Determination of In Vivo Clot Lysis

By *Alvin H. Freiman, Paul Ruegsegger, Irwin Nydick and Eugene E. Cliffton.* Sloan Kettering Division of Cornell University Medical College, New York.

The purpose of this presentation is to describe a direct in vivo method for measurement of the fibrinolytic activity of plasmin with radioactive fibrin.

Radioactive fibrin emboli of known size and

activity were injected into the right ventricle of anesthetized rabbits through a plastic catheter, these emboli subsequently lodging in the lung. Urine was collected by indwelling catheter every 30 minutes for 6 to 13 hours, and its radioactivity was determined in a scintillation counter. A control series of 10 rabbits given emboli containing a total of .05 mc. reached a steady base line of urinary excretory activity within 4 to 7 hours which was maintained throughout the period of observation. The urine of rabbits given fibrinolytic agents within 5 to 7 hours after embolization contained 3 to 10 times as much radioactivity as the untreated controls. Since each animal had reached a plateau of radioactivity in the urine prior to the administration of fibrinolytic agents, it served as its own control.

Autopsy studies were carried out to determine the uptake of radioactive materials in the lung and other organs. Uptake by organs other than the lung or liver was negligible. The treated animals showed 10–15% less residual pulmonary radioactivity than the controls.

Peripheral venous clots were formed with radioactive fibrin, and simultaneous local counting and urinary counting were done, demonstrating a close correlation between loss of local radioactivity and its appearance in the urine under treatment with plasmin.

The method described appears to provide a reliable index of the rate of clot dissolution in areas not accessible to external measurements. The results indicate a significant degree of clot lysis of pulmonary emboli as well as peripheral venous thromboses by plasmin.

### The Formation of Peripheral Clots in the Hypercoagulable State and Their Dissolution by Fibrinolysin

By *Alvin H. Freiman, Nils U. Bang, Paul Ruegsegger, Irwin Nydick and Eugene E. Cliffton.* Sloan Kettering Division of Cornell University Medical College, New York.

The purpose of this study has been the demonstration of the effect of fibrinolytic agents on experimentally induced peripheral arterial and venous clots, approximating those seen clinically in inflammatory thromboses. These clots were produced by a modification of the method that was originally described by Wessler. A temporary hypercoaguable state is produced by the infusion of serum followed by the production of local vascular stasis, resulting in nonadherent thrombus. The fat of these clots was followed by venography and arteriography at intervals of one hour, and by careful postmortem study of the vessel for residual clot.

*Results:* In a series of 21 dogs, ranging in weight from 12 to 40 pounds, clots were produced in one vein (9 dogs), one artery (1 dog), two veins simultaneously (6 dogs), one vein and one artery simultaneously (4 dogs), and one artery and two veins (1 dog). Control animals received 5% G/W by vein. In the treated animals fibrinolysin was administered beginning 1 to 8 hours after the production of clots, and was given for periods ranging from 1½ to 5 hours in doses sufficient to maintain a circulating fibrinolytic activity of 5 to 7 minutes. The fibrinolytic agents were administered systemically in 9 experiments, and locally in 12 experiments, with no untoward side effects.

Control arterial and venous clots showed no lysis for periods up to 12 hours.

With local administration of fibrinolysin 60% of clots were totally lysed, and 40% of clots were partially lysed. With systemic administration 47% of the clots were totally lysed, 47% were partially lysed and 6% showed no evidence of dissolution. Total lysis was achieved on the average in 2 hours and 15 minutes by local administration, and in 4 hours and 30 minutes by systemic administration. There appeared to be no significant difference in the time required by lysis of arterial as against venous clots.

Careful histologic studies of both treated and untreated clots have been carried out.

### Platelet and Fibrinogen Survival in Normal, Hypercoagulable and Hypocoagulable States

By *Edward Adelson, Jack J. Rheingold and William H. Crosby.* Department of Medicine, George Washington University School of Medicine, and Department of Hematology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. (Aided by a grant from the U. S. Public Health Service.)

We believe that coagulation goes on continuously in the normal. The process is kept in balance by anticoagulants which prevent the rate of formation of fibrin from exceeding the capacity of normal fibrinolysin. To measure the dynamics of this equilibrium we have compared the survival of some of the coagulation factors in the normal, the hypercoagulable and the hypocoagulable person and dog.

Platelet survival studies were carried out by

a method of in vivo $P^{32}$-tagging previously described by us. Fibrinogen survival has been studied by $I^{131}$-tagging. Hypercoagulability has been studied after adrenalin injections and in postoperative states. Hypocoagulability has been induced by dicumarol therapy.

We found that in 10 normal persons and dogs the half-life of platelets was 1½ to 3 days and the level of 10% platelet survival was reached in 6 to 14 days. Two hypocoagulable persons had platelet half-lives prolonged to 5 and 6 days. On the other hand, in 6 dogs made hypercoagulable by adrenalin injection the half-life was ½ to 2 days, and the level of 10% platelet survival was 2 to 6 days. In 4 postoperative dogs the half-life was ½ to 1½ days and 10% survival levels were reached in 1 to 3½ days. Similar results were obtained in 7 postoperative persons. Unlike platelet survivals, fibrinogen survival in persons and dogs did not appear to be altered by the hypercoagulable state.

We interpret these results to mean that in hypercoagulability there is an upset of the dynamic equilibrium in which the coagulation mechanism is normally maintained. However, only the first few steps in the chain of events which make up coagulation are involved in this upset. Equilibrium is re-established at some step prior to the fibrin-fibrinolysin level.

### The Coagulation Defect in Hemorrhagic Shock: An Expression of Intravascular Clotting

By *Rinaldo A. Turpini and Mario Stefanini.* Joseph S. Stanton Memorial Laboratories, Saint Elizabeth's Hospital, and Department of Medicine, Tufts University, Boston.

Thrombocytopenia, hypoprothrombinemia and fibrinogenopenia are frequently observed in hemorrhagic shock; their etiology remains fairly obscure. To clarify their mechanism, albino rabbits were bled until enough blood had been taken to account for ¾ of the original calculated blood volume. Various clotting factors were studied before, during and after bleeding. Events could be divided into three phases: (a) *early phase* (up to 60 min.): thrombocytosis, fibrinogenopenia, decreased activity of labile factor (V) and titer of complement were observed. The yield of generated thromboplastin increased, possibly because of the development of clot-accelerating factors in plasma and serum, transferable by transfusion into recipient animals. Antifibrinolysin activity was high and residual fibrinolytic activity low. (b) *Late stage* (lasting up to 12 hrs.): there was a progressive fall of platelets and of all clotting factors. Generation of thromboplastin was poor. Fibrinolytic and antifibrinolytic activities were respectively high and low. Deposits of fibrin, engulfing erythrocytes, were demonstrated in the hepatic and pulmonary vessels of animals sacrificed 2 hours after the initial bleeding. (c) *Recovery phase* (lasting up to 72 hrs.), characterized by a slow return to normal of all factors considered. Splenectomy, heparinization, ACTH and norepinephrine modified to a certain extent the described sequence of events.

*Conclusions:* (a) a complex hemostatic defect develops in the course of hemorrhagic shock. Laboratory and histologic findings indicate that this may be due to increased utilization of clotting factors through intravascular coagulation. The demonstration of a phase of early hypercoagulability in hemorrhagic shock and histologic findings confirm this hypothesis. (b) Activation of fibrinolysis accompanies the intravascular clotting. The time relationship between the two phenomena cannot be definitely established. Parallel studies indicate a similar sequence of events in hemorrhagic shock of man.

### Coagulation Changes, Systemic Toxicity and Fibrinolytic Activity Following Intravenous Plasmin (Fibrinolysin) Infusion in Humans

By *Kenneth M. Moser.* District of Columbia General Hospital, and Georgetown University Medical Center, Washington, D.C.

In experimental animals, intravenous infusion of plasmin (fibrinolysin), an active fibrinolytic material derived from human plasma, has consistently led to acute dissolution of intravascular thrombi with minimal attendant toxicity. The present study was designed to assess the systemic toxicity, coagulation changes and plasma fibrinolytic activity which follow intravenous plasmin infusion in humans.

Seventy-two infusions of highly purified human plasmin were carried out at 3 dosage levels: 30,000 fibrinolytic units (19 infusions); 40-50,000 units (28 patients); 69–90,000 units (25 patients). Multiple coagulation parameters, hematocrit, WBC, plasma fibrinolytic activity, temperature, pulse, blood pressure, electrocardiograms and urinalyses were followed serially for at least 24 hrs. following infusion.

Prothrombin time and content, plasma recalcification time and proaccelerin remained stable following infusion. Proconvertin and fibrinogen were transiently depressed, maximal losses aver-

aging less than 10% of baseline values. Hematocrit values were stable. A WBC elevation averaging 2800 was present 24 hrs. postinfusion. No parameter showed significant tendency toward greater abnormality as plasmin dosage was increased. No patient developed hemorrhagic phenomena following infusion, including 34 simultaneously receiving anticoagulant drugs.

Blood pressure showed no significant postinfusion deviations. A febrile reaction followed 44.4% of the infusion. Temperature elevation usually appeared 6 hours after start of infusion, reached a peak of 2.9 F. at 10 hrs. and returned to normal within the next 10 hrs.

Two patients developed electrocardiographic abnormalities following infusion (auricular fibrillation; transient T vector abnormality). Postinfusion urinalyses disclosed transient, mild albuminuria in 12 patients.

Enhanced plasma fibrinolytic activity developed following approximately 90% of the infusions. The intensity and duration of fibrinolytic activity paralleled the size of plasmin dose. Enhanced activity persisted 24 hrs. in 74% of patients receiving 69,000 units or more.

These studies suggest that intravenous plasmin can induce significant intravascular fibrinolytic activity in humans without prohibitive toxicity.

### Studies on the Thromboplastic Defect Resulting From Long-term Anticoagulant Therapy

By *Paul W. Boyles, Jane Harvey and Ulfar Jonsson.* Department of Medicine, University of Miami School of Medicine, and Miami Heart Institute, Miami, Florida.

Long-term anticoagulant therapy with dicoumarol or related compounds has been found to cause a defect in thromboplastin generation, in addition to the known effects on prothrombin conversion factors. A similar defect in thromboplastin generation has also been observed in patients with severe liver diseases.

The purpose of the present investigation was to try to identify the nature of the serum thromboplastic defect in these conditions. In order to do this, experiments have been performed to see if mutual correction could be obtained with sera with known thromboplastic factor deficiencies, such as PTC, Stuart factor and Hageman factor deficiencies, using the thromboplastin generation test of Biggs and McFarlane. It was found that the sera with specific deficiencies could only partially correct the thromboplastin generation test in these patients, while various combinations of these sera produced complete correction.

It is concluded from these experiments that the serum thromboplastic defect produced by long-term anticoagulant therapy and that seen in some cases of chronic liver disease is due to a deficiency of several factors which are essential to the generation of thromboplastin. The implications of these studies relative to the factors which contribute to normal production of thromboplastin will be discussed.

### The Relation of the Quick "Prothrombin Time" to Plasma Prothrombin Concentration in Patients on Long-term Coumarin Therapy

By *Sean M. Lavelle, H. S. Sise, Robert Becker and Betti Rose.* Tufts Medical Services and Anticoagulant Laboratory, Boston City Hospital, and Department of Medicine, Tufts University School of Medicine.

The prothrombin time of Quick is generally used for control of Coumarin therapy. But bleeding and thrombosis occur with a Quick time in the therapeutic range of 25–35 sec. We have shown that these complications are related to prothrombin concentration and that the Quick test does not reflect prothrombin concentration accurately. This study was done to find from a large number of measurements how often the Quick test is unreliable as a measure of prothrombin in the control of therapy in long-term patients.

The relation of the Quick prothrombin time (QT) to the prothrombin concentration (PC) in the plasma by the method of Owren was studied.

Both tests were done on 1,250 samples from 51 patients on phenindione over a total of 626 months. Samples were taken frequently when starting or resuming therapy, otherwise at monthly intervals. Dosage was aimed to give a PC of 10–24% without regard to QT. The QT was converted to the Quick value (QV) expressed in percent of normal from a standard dilution curve using adsorbed plasma.

The ratio for each sample was expressed as PC/QV. The overall mean per patient was 1.6 with a maximum of 3 and a minimum of 0.8. This ratio varied greatly between persons, and with time in the individual. Fluctuations were greater after change of dose, but were found on constant dosage over months, and with a constant QT. Variations tended to be cyclic over weeks, but were not related to time of year, Stuart factor level, viper venom time, or changes of reagents.

It was found that when the PC was in the correct range of 10–24%, then the QT fell outside the accepted therapeutic range of 25–35 sec. 45% of the time. When the QT was within the therapeutic range, the PC fell outside the correct range 30% of the time.

It is concluded that during anticoagulant treatment prothrombin time falsely represents the prothrombin concentration over 1/3 of the time.

### The Behavior of Reed-Sternberg Cells in the Bone Marrow

By *Robert J. Rohn and William H. Bond.* Department of Medicine, Indiana University Medical Center, Indianapolis.

Hodgkin's disease, a malignant granulomatous disorder of the reticuloendothelial system, is frequently associated with nonspecific alterations in marrow constituents and, on autopsy examination, may show patchy invasion of the bone marrow cavity with granulomatous tumor tissue. Because of the syncitial nature of the pathologic process, the attendant fibrosis, or other unexplained causes, it is rare to be able to demonstrate Reed-Sternberg cells on random needle aspiration of the marrow cavity of patients suffering from Hodgkin's disease.

Of the 92 patients with Hodgkin's disease seen in this Clinic, 4, who had been diagnosed by standard fixed tissue technics of lymph node biopsy, demonstrated numerous abnormal reticulum cells of the Reed-Sternberg type in bone marrow aspiration biopsy material during the course of their disease. Photomicrographic demonstration of tissues prepared with hematoxylin and eosin stain and Wright's stain have been prepared to outline the detailed comparative cytologic characteristics of these cells. Time-lapse, phase-contrast microcinematographic studies have been made to show the motility behavior of these cells and, in 1 patient, the effect of Chlorambucil upon the behavior of such cells.

### The Lipid Partition of 51 Patients with Multiple Myeloma

By *Bernard A. Sachs, Ethel Danielson and Paxton Cady.* Medical Division, Montefiore Hospital, New York.

In 1954, by paper electrophoresis and differential staining technics, we demonstrated the presence of an abnormal lipid-staining band associated with the abnormal protein and polysaccharide in 11 patients with multiple myeloma. The myeloma globulin band stains orange with oil red O and is not removed with Bloor's reagent which readily solubilizes alpha and beta lipoprotein from the filter paper strips. This study has been extended to further characterize the lipid-staining band and to include 51 patients with myeloma whose sera were also analyzed chemically for cholesterol, lipid phosphorus and total lipid.

The lipid-staining band was not altered by any lipid solvent which removed normal serum lipoprotein. Ethanol precipitates of serum carbohydrates (which contain protein), concentrated normal whole sera, normal gamma globulin and normal albumin, all stained orange with the oil red O after lipid extraction. Glucosamine did not take up the stain. It is concluded that the lipid-staining band associated with the myeloma beta or gamma globulin is present because of the high concentration of protein rather than to the presence of lipid.

The average serum cholesterol level was 161 mg.% (range 53–294 mg.%). 23 of 51 sera showed levels below 150 mg. %. The average lipid phosphorus was 8.2 mg. % (range 3.7–13.4 mg. %) with 6 values below normal, and total lipid, 771 mg. % (range 256–1,444 mg. %), with 11 values below normal. The average neutral fat was 405 mg. % (range 111–694 mg. %). The low serum cholesterol values were not necessarily related to the nutritional status of the patients.

### Anticomplementary Activity of Multiple Myeloma

By *Marvin L. Bloom, Sidney Shulman and Ernest Witebsky.* University of Buffalo School of Medicine, Department of Internal Medicine, Department of Bacteriology and Immunology, and Buffalo General Hospital.

Some instances of high titer anticomplementary activity (ACA) in multiple myeloma were reported in 1937 by Jersild. It was observed further that thermal potentiation (inactivation 55–65C.) of this activity was striking. However, several sera from patients, with no evidence of multiple myeloma, exhibited the same properties. The present authors observed a patient with high titer ACA, first documented in 1937 and again in 1949. At those times, no detectable indication of multiple myeloma was discovered. In 1955, he was found to have classic multiple myeloma and later died from this disease. Therefore, the symptom of high titer ACA in this case preceded diagnostic clinical changes by as long as 17 years. It is suggested that such a finding may herald

the presence of myeloma in other instances, many years before the clinical disease appears. Detection of such serum reactions is possible during routine serologic testing. Once such ACA becomes established, it appears to persist or even increase in strength. However, not all myeloma sera react in this way. The thermal potentiation of anticomplementary reaction, in correlation with striking changes in paper and optical electrophoretic observations, is under study. Thus far, by comparison with normal and dysproteinemic controls, high titer ACA appears to gain significance in the early diagnosis of multiple myeloma.

### Observations on Autotransplantation of Preserved Bone Marrow

By *N. B. Kurnick and Andrew Montano.* V. A. Hospital, Long Beach, California, and University of California Medical Center, Los Angeles. (Aided by a grant from the American Cancer Society and National Institutes of Health, U.S. Public Health Service.)

The discovery of a technic for preserving viable bone marrow indefinitely by slow freezing in glycerol permits pre-irradiation collection of bone marrow from patients who require extensive radiotherapy. Some neoplasms, such as seminoma, are potentially curable by radiotherapy even in the face of demonstrable metastases. However, therapy must often be discontinued because of severe bone marrow depression by the irradiation. Autotransplantation of bone marrow would be expected to repopulate the marrow and thus permit completion of the radiotherapy. No problems of immune-reaction and rejection of the transplant, such as attend homotransplants, need be anticipated.

We have collected and stored bone marrow from several patients and have completed the experiment twice in one. This patient with seminoma and embryonal carcinoma with pulmonary metastases received the planned course of 2,000 r to the entire torso over a period of 2 months despite severe hematopoietic depression. Rapid recovery of the bone marrow and peripheral blood followed intravenous infusion of his stored marrow. Six weeks later, after his bone marrow had returned to normal, bone marrow was again aspirated and stored. This was reinfused 5 weeks later following hematopoietic depression by a second course of radiotherapy for extradural and pulmonary metastases. The results indicate the procedure is safe and may be resonsible for the favorable hematopoietic recovery observed.

### Observations on Chimpanzees after Whole Body Radiation and Homologous Bone Marrow Treatment

By *Harvey Rothberg and Joseph H. Akeroyd.* Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C.

Three fasting 40–50 pound chimpanzees were each given 900 r of whole body gamma radiation from a 3.5 mev Van de Graaff machine. Thirty-three hours later, each animal was given 15–21 billion nucleated homologous bone marrow cells intravenously, the entire amount which could be obtained from 4 long bones and the vertebral column of another chimpanzee of similar size. All 3 animals developed extreme leukopenia and thrombocytopenia, reaching a nadir by days 10–13, and there was a progressive anemia. One animal died of Klebsiella bacteremia and shock on day 16. A second died of internal bleeding on the 19th day. The 3rd animal had a transient reticulocytosis on day 17, and a definite increase of leukocytes with a secondary reticulocytosis beginning on day 27. She now survives after 60 days, having recovered to essentially normal hematologic values. A partial alopecia and marked dryness of the skin were noted 3 to 4 weeks following radiation, and these findings continue; otherwise the surviving animal appears entirely normal. It was unfortunately not possible to study survival of the transplanted cellular elements in this female chimpanzee. However, circulating antitoxins in this animal remain qualitatively like those she produced prior to the radiation, and do not resemble those of the marrow donor.

There was surprisingly little gastrointestinal symptomatology. All animals appeared sick and dejected for several hours following radiation. There was no vomiting. They had a mild nonbloody diarrhea on the 2nd and 3rd day after radiation, and occasional loose stools thereafter. This suggests that a higher dose of radiation could be tolerated, and might be preferable in subsequent experiments on bone marrow transplantation.

# BLOOD PROTEINS

## Studies on Acquired Hypogammaglobulinemia: Evidence of Antibody Formation and One Case with Bronchial Asthma

By *Theophilus S. Painter, Jr. and Donald R. Korst.* Radioisotope and Medical Service, V. A. Hospital, and Department of Medicine, University of Michigan Medical School, Ann Arbor.

Two patients with acquired primary hypogammaglobulinemia received various antigens, and their response to therapy was studied.

Sensitive indicators of antibody–antigen reaction such as $Cr^{51}$-labeled heterologous red blood cells in vivo demonstrated ability of these 2 patients to respond to a challenge of incompatible blood types. In one patient, ABO antibodies were apparently exhausted by 20 ml. of incompatible blood given 4 days before the 2nd injection of labeled cells. In another patient external counting over the spleen after a repeat test indicated more splenic sequestration following prior infusion of opposite type cells. The cell survival time was significantly shorter in the 2nd run. The disappearance rate of immune human gamma globulin (IHG) was determined 2 ways; by daily serum paper electrophoresis and prior labeling with radioiodine ($I^{131}$). Large single intravenous doses were used on 3 occasions without serious reactions. The maintaining of normal gamma globulin levels was the same with the i.m. or the i.v. route of administration. A response was obtained with adenovirus vaccine.

The occurrence of infectious asthma in a patient with hypogammaglobulinemia has not been previously reported. Extensive immunologic testing as well as therapy observations have been made in this patient over a 5-year period. This syndrome in adults should be referred to as hypogammaglobulinemia, and the variation in antibody responsiveness among patients recognized. Indications of antibody formation in 2 adults with acquired hypogammaglobulinemia are demonstrated by adenovirus vaccine and $Cr^{51}$ labeling of heterologous erythrocytes.

## Phagocytosis of Cryoglobulin by Leukocytes

By *Paul Heller, Vincent Yakulis, Sheldon E. Krasnow and Michael L. Glick.* Medical Service and Research Laboratory, V. A. Westside Hospital, Chicago, and Department of Medicine, University of Illinois College of Medicine. (Aided by a grant from the Hematology Research Foundation.)

In the L.E. cell preparation from a patient with severe cryoglobulinemia, polymorphous crystals were found within leukocytes in addition to the amorphous inclusion body previously described by Volpe. This led to a systematic search for these crystals in other patients.

The L.E. test was performed with blood samples from more than 100 patients. Aliquots of clotted, oxalated, and heparinized blood samples were incubated for 1 hr. at 25 C. or 37 C. and the incubation continued for 1 hr. at 5 C. This sequence was reversed with other aliquots. The serum or plasma of patients were also incubated with cells from normals. Other methods used for the physicochemical and immunologic characterization of the cryoglobulin were: "Cryocrit"-determination, paper electrophoresis, ultracentrifugal analysis, and the agar-gel-diffusion technic of Ouchterlony.

Intracellular crystals were found only when cryoglobulinemia was also demonstrated in the traditional manner. In no case was cryoglobulinemia demonstrable in the absence of crystals.

Extracellular crystals were observed if incubation took place at 5 C., but at this temperature no intracellular crystals were seen. These were present only in the specimens which were incubated at 37 C. or 25 C., and their formation was not significantly enhanced by subsequent exposure to 5 C. Repeated washings of the buffy layer did not result in diminution of the number of intracellular crystals. The protein nature of these crystals was confirmed immunologically by use of the agar-gel-diffusion technic with a lysate of crystal containing leukocytes as one of the antigens.

Cryoglobulin probably is phagocytized by leukocytes during incubation, and crystallization occurs intracellularly. It appears that the physicochemical conditions within the leukocyte permit crystallization of cryoglobulin at higher temperature than extracellularly.

Apart from its theoretical significance, the described phenomenon offers a sensitive and apparently specific test for the demonstration of cryoglobulin.

### The Idiopathic Hypoalbuminemic Syndromes: Differentiation of Excessive Destruction from Deficient Production

By *J. L. Steinfeld and T. Waldmann.* Metabolism Service, General Medicine Branch, National Cancer Institute, Bethesda.

Patients with idiopathic hypoalbuminemia have no demonstrable hepatic, renal, endocrine, cardiac or gastrointestinal disease. In the study of a patient with idiopathic hypoalbuminemia we found a rapid turnover of $I^{131}$ albumin with excretion of large amounts (10 to 100 times normal) of the label in the stool. While the patient studied had few of the symptoms associated with regional enteritis, subsequent study of patients with proven regional enteritis or ulcerative colitis showed that the hypoalbuminemia is largely a result of loss of body albumin into the bowel rather than failure of albumin synthesis by the liver, since synthesis of albumin in such patients may proceed at twice the normal rate.

In a study of 2 other patients with idiopathic hypoalbuminemia the $I^{131}$ content of the stool was not increased as in the patients with ulcerative intestinal disease. Rather, one of the patients had an albumin turnover of less than ½ normal and the other had an increased $I^{131}$ albumin degradation rate, equally unexplainable. Review of the clinical records in these patients and of 27 reported cases that we could find in the medical literature, whose reports were detailed enough to rule out the common etiologies, revealed at least two newly recognized syndromes of which idiopathic hypoalbuminemia with or without edema is a presenting feature. These two syndromes, to be called (a) hypercatabolic idiopathic hypoalbuminemia and (b) ananabolic idiopathic hypoalbuminemia, have distinguishing clinical characteristics.

## CARDIOVASCULAR SYSTEM

### Dissociation of Electrocardiographic Activity from Myocardial Contractility by Ionic Alterations

By *Robert M. Kohn.* University of Buffalo, Chronic Disease Research Institute, Buffalo, New York. (Aided by a grant from the Erie County Heart Association.)

Electrical and mechanical events of the cardiac cycle may be reversibly separated by altering the calcium concentration (Mines, 1913). This effect has been further investigated and the effect of other cation alterations observed.

Isolated rabbit hearts were perfused with Chenoweth's solution in an Anderson apparatus. Direct electrocardiograms and intraventricular pressures were recorded. When the control perfusate was replaced by one with no calcium, the heart stopped in diastole and mechanical activity disappeared, although electrocardiographic evidence of activity continued essentially unchanged. The atria continued to beat mechanically longer than the ventricles. Mechanical activity resumed immediately after perfusion with a solution containing calcium.

Atrioventricular block was the commonest arrhythmia observed when neither calcium nor magnesium was present in the perfusate, especially when the coronary flow was slowed by raising the right atrial pressure. Magnesium in the perfusate tended to normalize the electrocardiogram. Ouabain had no effect on contractility of a heart stopped by calcium depletion.

Hearts stopped by calcium depletion were refractory to direct electrical stimulation. Although they could be shocked into electrical fibrillation, mechanical activity was not seen until calcium was added to the perfusate; at this time mechanical fibrillation appeared.

Administration of potassium stopped both electrical and mechanical activity. Ethylenediamine tetra-acetic acid, which binds all cations, caused either electrical standstill or fibrillation.

These experiments suggest that calcium has at least two different sites of action in the heart, one on the action potential and the other on the contractile process. It is possible that calcium is necessary for the electrical impulse to bring about muscular contraction. Magnesium is capable of partially replacing calcium in the membrane (electrical) effect but not in the process of contraction.

### Potassium Servomechanism Regulating Cardiac Work, Role of Potassium in Myocardial Disease and Angina Pectoris

By *Richard S. Gubner.* Bureau of Medical Research, Equitable Life Assurance Society of the United States, New York.

In previous work it was observed that calcium immediately augments cardiac force. Since the myocardial cell is poorly permeable to Ca, this effect is attributed to a cell surface action, i.e., a sluice effect of Ca promoting efflux of potassium (decrease of K intracellular/extracellu-

lar ratio) which enhances contractility. Digitalis and epinephrine act similarly.

Muscular contraction is attended by a pulsatile potassium efflux, maximal at the time of the T wave. K efflux is augmented when cardiac work load and heart rate are increased, and it is suggested that this acts as a autoregulatory mechanism to increase cardiac contraction. Heightened T wave amplitude with exercise reflects augmented K efflux.

Physiologically cardiac output increases via augmented contractility without greater diastolic size. In K depletion, ageing, myocardial disease, or with excessive work load, the K efflux mechanism is taxed, depleting intracellular K. With failure of this primary mechanism, compensation is then effected by increased filling (cardiac enlargement) permitting the Starling law mechanism to become operative.

In coronary disease myocardial cellular K loss is markedly accentuated by ischemia, and extracellular K is further excessively elevated since it is not washed away rapidly due to poor blood flow. Such K changes in ischemic areas favor contracture which may be a factor in pain. In addition, the greatly increased K in the coronary veins appears to contribute to anginal pain. This is suggested by the observation that pain and venoconstriction are produced in veins by infusion of KCl at rates exceeding 2 mEq./min. Conditions which produce angina pectoris, such as ischemia, work, epinephrine, cold, all deplete myocardial K and increase coronary venous K.

### Cation Flux of the Left Ventricle: Influence of Increased Work Induced by Sympathomimetic Catechol Amines on Oxygen and Carbohydrate Utilization

By *Jerry E. Schmitthenner, Ivan Forte, A. Jane Williams, Cecilia Riegel and J. H. Hafkenschiel.* Cardiopulmonary Unit, Lankenau Hospital, Philadelphia. (Aided by grants from the National Heart Institute, Heart Association of Southeastern Pennsylvania and the National Research Council.)

To ascertain the effects on myocardial oxygen utilization, carbohydrate uptake, and cation flux in healthy and ill subjects when heart work increases, this study was made in young pentobarbitalized dogs, as a preliminary to clinical physiologic experiments. Equidosage norepinephrine (NA) and epinephrine (A) infusions over 30 min. were given after measurements were made of coronary flow (nitrous oxide), cardiac output, heart rate, mean arterial pressure and arteriovenous oxygen differences of pH, oxygen, hematocrit, glucose, lactate, pyruvate, sodium, potassium, calcium and magnesium. Repetition of the observations during the last 10 min. of the infusion afforded in each experiment 2 measurements of left ventricular work, oxygen, carbohydrate and cation "usage." Each dog was studied 6 times at monthly intervals, there being 2 saline (PSS) infusions, 2 NA (3 μg./Kg. per min.) and 2 A, allowing mean differences induced by catechol amines to be compared with mean differences during PSS.

NA increased left ventricular stroke work 200%, A, 300%. NA augmented coronary flow 13%, A, 29%. Both increased oxygen uptake with greater oxygen extraction. Mean coronary venous oxygen tension was not reduced because of increased hemoconcentration and concordant pH changes. Both NA and A reduced glucose uptake; lactate utilization decreased with NA and was unchanged by A. NA increased sodium "usage," but release followed A. Both NA and A increased left ventricular potassium utilization. Calcium and magnesium uptake were unchanged. These observations suggest: (1) that increased left ventricular stroke work and presumably contractile force can be accomplished without a greater extraction of lactic acid from the arterial blood, fatty acids (not measured) being the probable energy source; (2) that the energy transformations are associated with an uptake of potassium; and (3) only during A infusion was a concomitant sodium release observed.

### The Effects of Intravenous Aminophyllin upon Coronary Hemodynamics and Myocardial $O_2$ and $CO_2$ Metabolism of the Normal Person

By *George M. Maxwell, Douglas H. White, Jr., Charles W. Crumpton, George G. Rowe and Cesar A. Castillo.* Cardiovascular Research Laboratory and Departments of Pediatrics and Medicine, University of Wisconsin, Madison.

Contradictory opinions exist concerning the action of xanthines upon the coronary vasculature. This study was done to explore the effects of aminophyllin in normal persons.

Fourteen normals were studied. Control cardiac output (Fick) and coronary blood flow ($N_2O$ Fick) were measured. Aminophyllin (250 mg.) was given into the coronary sinus catheter for over 20 min. and the coronary blood flow and cardiac output re-measured. Appropriate control and experimental pressures were recorded.

Respiratory exchange increased significantly and was associated with an increase in total body $O_2$ extraction and $CO_2$ production. Mean values (control and drug) for heart rate were 80 and 78/min., for MABP 96 and 96 mm. Hg., and cardiac index 4.0 and 3.5 L./min./$M^2$. Calculated total peripheral resistance increased. Right atrial, end-diastolic right ventricular, mean pulmonary artery, and pulmonary capillary pressures all declined significantly. The coronary blood flow decreased from 79 to 69 cc./100 Gm./min. ($p < 0.01$). Thus the coronary vascular resistance rose as a factor of the unchanged MABP associated with the reduction in coronary flow. The coronary sinus $O_2$ content decreased by 18% ($p < 0.05$). Thus the myocardial $O_2$ consumption was maintained in the face of a reduced flow by an increase in the $O_2$ extraction.

Under the conditions of these experiments, 250 mg. of aminophyllin administered intravenously to normal man resulted in a significant increase in total peripheral and coronary vascular resistances. Vasodilatation was observed to occur only in the pulmonary vascular system.

### Influence of the Rate of Coronary Plasma Flow on the Extraction of $Rb^{86}$ from Coronary Blood

By *W. D. Love and G. E. Burch.* Department of Medicine, Tulane University School of Medicine, and Charity Hospital of Louisiana.

Indirect evidence from previous studies indicates that the rate of coronary plasma flow (CPF) largely determines the rate of increase in myocardial radioactivity during an intravenous infusion of $Rb^{86}$. In the present study the relationship of myocardial $Rb^{86}$ uptake and CPF has been measured directly.

In 19 dogs the rate of outflow of blood from a cannula in the coronary sinus was measured volumetrically at intervals during an intravenous infusion of $Rb^{86}$. The amount of myocardium drained by the coronary sinus cannula was calculated from the total amount of $Rb^{86}$ extracted from the blood which flowed out of the cannula and the final myocardial $Rb^{86}$ concentration measured at sacrifice. To determine the $Rb^{86}$ concentration of the myocardium at the time of successive measurements on each dog, the product of CPF and arteriocoronary sinus plasma $Rb^{86}$ difference was accumulated serially. L-norepinephrine was administered to some animals to produce high rates of CPF.

Myocardial $Rb^{86}$ extraction was found to be logarithmically related to CPF in the range from 15–200 ml./100 Gm./min. (log % $Rb^{86}$ extracted = 1.93 − 0.00175 CPF). There was approximately 8.5% fall in $Rb^{86}$ extraction for each 10% rise in myocardial $Rb^{86}/K$ : plasma $Rb^{86}/K$. No direct effect of l-norepinephrine on $Rb^{86}$ extraction was detected.

Applying these results to man, a 50% increase or decrease in the average CPF would be expected to produce respectively a 38% rise or 44% fall in $Rb^{86}$ uptake. There would be approximately 10% fall in the extraction of $Rb^{86}$ due to rising myocardial $Rb^{86}$ content by the end of 7 min. of $Rb^{86}$ infusion.

These relationships indicate that clinically valid estimates of coronary plasma flow can be made without cardiac catheterization by measuring myocardial $Rb^{86}$ uptake, provided that an external monitor can be devised which will measure myocardial radioactivity accurately.

### The Effect of Coronary Arterial Denervation on Coronary Hemodynamics

By *Norman Brachfeld, R. Grier Monroe, Elin Alexanderson and Richard Gorlin.* Medical Clinic, Peter Bent Brigham Hospital, Boston, and Department of Medicine, Harvard Medical School.

Prior studies under a variety of conditions have demonstrated that myocardial $O_2$ extraction is near maximal; decreases in cardiac oxygen demands are met by decreases in coronary flow and not by decreases in extraction. 12 thoracotomized dogs were subjected to periaortic and coronary neurectomy by dissection and phenolization. Coronary flow ($N_2O$ technic), myocardial $O_2$ consumption, A-V $O_2$ extraction, left ventricular work ($LV_W$), heart rate and diastolic coronary vascular resistance (CVRd) were measured before and after denervation. In 8 animals coronary sinus $O_2$ saturation increased from 4.0 to 8.5 V.P.C; A-V $O_2$ difference narrowed 4.0 V.P.C; coronary flow remained unchanged or increased reciprocally depending on whether heart rate, $LV_W$ and therefore $O_2$ consumption decreased or remained unchanged. CVRd showed variable change. However, when compared to the theoretically predicted CVRd (assuming a fixed A-V $O_2$ under the same hemodynamic circumstances in innervated preparation) the observed resistance was 30% less than that theoretically calculated. In the intact dog, change in $O_2$ demand is met by variation in flow. In the denervated preparation apparently arterioles show greater vasodilatation than seen normally at a given $O_2$ consump-

tion, with consequent narrowing of A-V $O_2$ extraction. These studies indicate neural control of coronary flow apart from that occasioned by alterations in hemodynamic and metabolic environment.

### Histochemical Study of Hypertrophied Heart Muscle

By *Andrew Kerr, Jr. and Edward J. Kollar.* Department of Medicine of State University of New York, Upstate Medical Center, and V. A. Hospital, Syracuse.

Following the method of Hajdu and Beznák (1944), metal rings were placed about the ascending aorta of adult white rats. Allowing 4 weeks for the development of cardiac hypertrophy, animals were sacrificed and hearts were weighed. In 7 animals with hypertrophy of the heart and in 9 controls, histochemical technics were used to evaluate enzyme activity.

Stains were used to estimate activity related to: succinic dehydrogenase, ribonucleic acid, desoxyribonucleic acid and phosphatases. Succinic dehydrogenase activity was increased in 6 hearts of the 7 animals with cardiac hypertrophy. In the 9 controls a comparable increase of enzyme activity was noted in only 2 animals. No difference was demonstrated between the hypertrophied hearts and the normal hearts for phosphatases, for ribonucleic acid or for desoxyribonucleic acid.

Metabolic activity, as measured by succinic dehydrogenase, appears increased in experimental cardiac hypertrophy.

### The Physiologic Effect of Heat Stress in a Steel Mill

By *Amasa B. Ford, David J. Turell and Herman K. Hellerstein.* Department of Medicine, University Hospitals of Cleveland, Ohio.

Thirty-two normal men and 20 men with recognized heart disease were studied during routine work in a steel mill. Heat stress was measured on the scale of Belding and Hatch as a balance between heat load (radiation, convection and metabolism) and ability of the body to lose heat (radiation, convection and evaporation). A heat stress index of 100 represents any combination of circumstances in which heat load equals maximum possible loss. Three types of working environment were recognized. *A steady high* heat exposure (open hearth furnace, pouring platform, hot metal inspection) was characterized by an average heat stress index of 55–86. *Intermittent high* heat stress (blast furnace, maintenance areas, foremen) had average indices of 9–34. *Low* heat stress areas (control operators) gave indices of 0–22. Certain individual workers were exposed to heat stresses of over 300 for totals of an hour or more out of the work shift.

High heat stress was associated with a significant increase in working pulse rate and recovery pulse sum (total pulse beats during first 3 min. of recovery). The presence of compensated heart disease could not be shown to affect working pulse rate or recovery pulse sum. The correlation between pulmonary ventilation and energy expenditure (as measured by oxygen consumption) was not influenced by heat stress or the presence of heart disease. A decrease in blood pressure was observed in certain individuals working in hot areas.

### Left Ventricular Work in Normal and Enlarged Human Hearts

By *Orland Baker and Carleton B. Chapman.* University of Texas Southwestern Medical School, Dallas. (Aided by Dallas Heart Association.)

Instantaneous left ventricular volume curves were recorded in human beings by a biplane cineangiofluorographic technic. Two images, RAO and LAO, were recorded simultaneously at 15 frames/sec. after the injection of radiopaque medium. Aortic pressure curves were recorded at the same time. From the biplane film strips paired tracings of the left ventricle were made of each frame. Measurement of ventricular diameters at 1.0 mm. intervals were then made from the tracings and, by an application of Simpson's parabolic rule, instantaneous ventricular volume was calculated. Validation of the method of volume calculation was done by applying the technic to various models of known volume, which shows the method to have a random error of less than 10%.

By use of the technic, left ventricular function was studied in a normal man and in a patient with marked left ventricular enlargement who had recently been treated for congestive failure. Work loops and total ventricular (stroke) work were markedly different in the 2 subjects. In the normal subject, total stroke work was 10,512 Gm. cm. (0.5% kinetic, 99.5% potential). In the abnormal subject, total stroke work was 29,454 Gm. cm. (1.3% kinetic, 98.7% potential). Stroke vol-

ume in the normal was 84 cc., and in the abnormal 89 cc. Corresponding minute volumes were 7.2 and 10.7 L./min.

The work demonstrates the feasibility of studying ventricular function with precision, and the cases presented demonstrate the fact that an overloaded, grossly enlarged, recently failing but compensated ventricle may generate far more power than the normal ventricle.

### Prediction of the Duration of the Dynamic Phases of the Cardiac Cycle by Intracardiac Phonocardiography

By *Howard L. Moscovitz, Ephraim Donoso and Ira J. Gelb.* Division of Cardiology, Department of Medicine, Mt. Sinai Hospital, New York.

The duration of the mechanical events of the cardiac cycle has been classically measured by analysis of multiple simultaneous pressure recordings. While this is possible in man, the procedures required to obtain direct pressure pulses are complicated and result in discomfort if not morbidity to the patient. Measurement of these dynamic events by an acoustic method therefore would have obvious advantages.

The chief factor contributing to equivocal results in predicting the length of the dynamic phases from a conventional phonocardiogram is superimposition of sounds from neighboring valve areas upon the vibrations produced by the valve to be studied. The analysis would become far simpler if sounds from the opposite side of the heart could be excluded. Correlation studies of acoustic and mechanical events would appear to be advanced by the detection of sounds from within the heart at their site of origin, a development now possible with the use of an intracardiac microphone.

A ceramic microphone sealed in the tip of double-lumen cardiac catheter was passed into the various cardiac chambers both in patients with heart disease and in dogs with surgically produced valve lesions. Intracardiac sounds were recorded simultaneously with multiple pressure pulses. The duration of the individual phases of the cardiac cycle was then measured from the mechanical and from the acoustic records and the results plotted against each other. It was possible to predict the length of any of the dynamic phases from a well written intracardiac phonocardiogram with an accuracy of ±0.02 second. For some of the phases, such as total ejection, protodiastole, or total diastole, the error was smaller, averaging less than 0.01 second.

### Selective Pulmonary Arteriography: Application in Congenital and Acquired Heart Disease

By *Seiichi Shimomura, W. James Guthrie and A. L. Loomis Bell, Jr.* Cardiopulmonary Laboratory, St. Luke's Hospital, New York City. (Aided by a grant from the New York Heart Association.)

A clinical method for visualizing radiographically the smaller pulmonary arteries and arterioles has been devised. At right heart catheterization, the cardiac catheter is wedged into a pulmonary artery and an x-ray exposure made after injection of 3 cc. of 70% Urokon, using nonscreen film at 90–110 kv. 300mA, and 1/30 sec. exposure.

This technic has been applied in the study of 25 cases with cardiac defects showing hemodynamic patterns of: (1) normal pulmonary artery pressures and increased pulmonary flow; (2) high pulmonary artery pressure and increased pulmonary blood flow; (3) high pulmonary artery pressure and decreased pulmonary flow; and (4) increased pulmonary artery pressure due to increased left atrial pressure. The arteriograms of these patients have been correlated with the hemodynamic studies and with lung specimens obtained at surgery or autopsy.

The normal pulmonary arteriogram shows abundant arborization of small arteries of 200–2000 micra, with filling of smaller arterioles and capillaries producing distinctive background markings. The abnormal pulmonary arteriogram of patients with pulmonary hypertension and balanced or right-to-left shunt, shows sparcity of arborization of these small pulmonary arteries, abrupt termination of 800–1200 micra arteries, or abrupt narrowing of these arteries into fine, tortuous channels without normal capillary filling. The cases with normal pulmonary artery pressure and either low or high pulmonary flow may show no changes as compared with the normal. The abnormal arteriogram shows an excellent correlation with the histologic picture of intimal proliferation and occlusive changes seen in pulmonary arteries of 300–1000 micra diameter in those patients with high pulmonary artery pressure and decreased pulmonary blood flow. The arteriograms of patients with pulmonary artery hypertension due to left heart obstruction may

show decreased arborization of an intermediate degree.

These preliminary studies indicate that selective pulmonary arteriography may have real value in demonstrating radiographically occlusive intimal changes in certain cases of secondary pulmonary hypertension. The method should prove useful in selecting patients with congenital cardiac defects in whom correction of the defect is feasible.

### The Measurement of Cardiac Output by the Thermodilution Method

By *Elliot Rapaport and Sigmund G. Ketterer.* Cardiopulmonary Laboratory, Mount Zion Hospital, San Francisco.

The thermodilution method for measurement of cardiac output was studied in normal dogs.

A bead thermistor was mounted in the distal end of a double lumen cardiac catheter, the proximal end of which opened 15 cm. from the tip. The output of the thermistor was fed into a D.C. Bridge Balance, and changes in current were recorded with a conventional oscillograph.

The thermistor tip was placed in the main pulmonary artery. Following the determination of a direct Fick cardiac output, 2 to 4 cc. of Ringer's solution varying in temperature from 1° to 4° C. were injected into the right atrium through the proximal lumen, and the resultant time-temperature dilution curve recorded. The thermodilution output was calculated by the indicator dilution technic using the formula suggested by Fegler.

Twelve determinations have been performed. The average of the ratios of the cardiac outputs calculated by the thermodilution method to the direct Fick outputs was 1.08 (s.d. = .24, $p > .2$). A prolonged tail to the downstroke of the thermodilution curve was frequently seen, and the largest deviations between the two methods occurred when this was most pronounced. This terminal delay in the downstroke is thought to arise from cooling of the atrial and ventricular wall with subsequent temperature exchange and recooling of undiluted blood continuing to enter the heart. However, the possibility of incomplete mixing as a factor arises due to the short distance between injection and temperature detection; the assumption that turbulence produced by right ventricular contraction would eliminate this problem may not be valid.

It is concluded that a significant correlation exists between the two methods, although the relatively large standard deviation precludes present routine use of this thermodilution technic for determination of cardiac output.

### Estimation of Cardiac Output Utilizing Radioiodinated Serum Albumin and Precordial Detection

By *Bernard F. Schreiner, Jr., Frank W. Lovejoy, Jr. and Paul N. Yu.* Department of Internal Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. (Aided by a grant from U.S.P.H.S.)

The estimation of cardiac output utilizing precordial dilution curves obtained following the rapid intravenous injection of RISA (radioiodinated serum albumin) was carried out consecutively 65 times in 31 patients with a variety of cardiopulmonary diseases. The apparatus consisted of a 1¾ inch (diameter) x 1⅛ inch (length) thalliated sodium iodide crystal coupled through a photo-multiplier tube to a count rate computer, the output of which was recorded at one or 2-second intervals on a one milliampere galvanometer type recorder. Curves were recorded with the crystal, collimated with 4 inches of lead, placed over the base of the heart. Individual doses of 6–80 μc. (RISA) were employed with a total dosage of less than 160 μc. (RISA) needed for repeated determinations.

Forty-four precordial estimates of cardiac output were compared to simultaneously obtained values from direct arterial curves in 24 patients. Four-fifths of the precordial measurements agreed within ±11% with the arterial curves, and in all but one of the precordial curves the agreement was within ±20%.

Twenty-eight precordial flow measurements were compared to nearly simultaneous Fick determinations in 15 patients. Three-fourths of the precordial measurements agreed within ±11% with the Fick method, and in all but one instance the agreement was less than ±22%.

Duplicate precordial flow estimates were carried out in 31 patients. Seven-tenths of the duplicates agreed within ±11% and the majority of the remainder within ±20%.

It is concluded that the method employed is reproducible and gives adequate estimates of blood flow when compared to more conventional methods.

### Measurement of Cardiac Output by a Direct Writing Surface Scintillation Technic

By *Richard Gorlin, Colin MacLeod and Pierre Bopp.* Medical Clinic, Peter Bent Brigham Hospital, Boston, and Department of Medicine, Harvard Medical School.

Because of the need for elaborate catheterization or arterial cannulation technics for measurement of cardiac output, this circulatory parameter has rarely been available as a clinical method. The externally recorded precordial dilution curve following intravenous injection of 10–30 μc. radioactive serum albumin may be analyzed for cardiac output according to the method of MacIntyre et al., utilizing circulating blood volume. Injections at different sites between antecubital vein and pulmonary artery have been made in 15 patients and 10 dogs. Dilution outputs were measured simultaneously by direct arterial blood sampling, with a correlation, R of 0.74 with the surface method. The surface outputs gave reproducible values when repeated, regardless of injection site. The curves were bactrian in type with first right then left heart peaks. Intraventricular transit time from peak to peak normally averaged 7.3 seconds and regional blood volume estimated from this time was 380 cc./M.$^2$ B.S.A. or 13% of blood volume.

Serial studies have shown: (1) in 4 patients that coronary shock is a low output state and that output rises with digitalization; (2) some cases of postoperative shock are accompanied by low blood pressure, normal output and inadequate systemic vasoconstriction.

### Evidence Against Significant Extravascular Distribution of $I^{131}$-HSA (Human Serum Albumin)

By *Frank A. Finnerty, Jr., John Tuckman and Joachim H. Buchholz.* Cardiovascular Research Laboratory, Georgetown Medical Division, District of Columbia General Hospital.

Detailed simultaneous early dilution curves of chromium-labeled red cells (RBC-$Cr^{51}$) and $I^{131}$-HSA were obtained in the hope of clarifying the discrepancy between total body and large vessel hematocrit.

Ten patients without cardiovascular disease received intravenously RBC-$Cr^{51}$ and $I^{131}$-HSA mixed in the same syringe. Dilution curves for each isotope were determined from 36 arterial samples drawn during the first 20 minutes and 10 samples drawn during the remaining 100 minutes.

These data revealed: (1) dilution curves of both isotopes were composed of 3 phases, an initial steep phase (I) lasting 40 to 60 seconds, a less steep phase (II) lasting 15 to 20 minutes and a final phase (III) with the slightest slope; (2) time periods and slopes of each phase of both isotopes were similar for each patient; (3) at least 80% of the 30-minute dilution of both isotopes occurred by the end of phase I; (4) in each patient, at the end of phase I, the ratio of the total body hematocrit to the large vessel hematocrit was approximately 0.9 where it remained throughout the experiment.

If one accepts the assumptions that (1) RBC-$Cr^{51}$ do not leave the intravascular compartment during the first 20 minutes and (2) a significant amount of $I^{131}$-labeled protein does not leave the intravascular compartment during the first 60 seconds, then it is logical to conclude from the data presented that (a) the discrepancy between the total body hematocrit and the large vessel hematocrit present at the end of phase I is due to the invasion of an intravascular network with an average hematocrit below the large vessel hematocrit; (b) the parallel slopes and similar time periods of phase II indicate that the additional network invaded by both isotopes is also intravascular with the same average hematocrit as in phase I; (3) a marginal plasma layer (Whipple) cannot be significant; and (4) phases I and II of both isotopes represent true intravascular mixing and hence the extrapolation of phase III of $I^{131}$-HSA represents a reliable method for determining true plasma volume.

### Comparison of Central Blood Volume Measurements in Dogs, Using Stewart-Hamilton Formulae and $Cr^{51}$-Tagged Red Blood Cells

By *Robert C. Schlant, William L. Kraus, Charles B. Moore, Florence W. Haynes and Lewis Dexter.* Departments of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston. (Aided by grants from the Life Insurance Medical Research Fund and the National Heart Institute.)

Indicator (T-1824) dilution curve measurements of central blood volume from right atrium to ascending aorta were made in 22 open-chested dogs which earlier had had their red blood cells tagged with $Cr^{51}$. Prior to obtaining the dye curve, the azygos vein was ligated and tapes put loosely around the venae cavae close to the right atrium. T-1824 was injected through a catheter with its tip in the superior vena cava below the level of the tape and just above the right atrium. During actual injection, both cavae were momen-

tarily occluded. Multiple one-second samples were obtained from just above the aortic valve and analyzed for dye concentration. Immediately after the dye curve had been obtained, both cavae, the aorta and pulmonary artery were simultaneously occluded. Central blood volume ($CBV_{T\text{-}1824}$) was computed from the T-1824 dye curve, using the Stewart–Hamilton mean transit time–cardiac output formulae. The heart and lungs were removed, the tissues blended, and the total radioactivity of the homogenate determined to calculate the actual central blood volume ($CBV_{Cr^{51}}$). The two measurements correlated well ($R = .88$), with the indicator-dilution measurements systematically being slightly greater (average 11%). The values were best related by the following regression equation:

$$CBV_{T\text{-}1842} = 3.25 + 1.10\ CBV_{Cr^{51}}$$

The results substantiate the use of mean circulation time–cardiac output formulae to measure central blood volume.

### Observations on the Reduction of Stroke Volume and Central Venous Pressure Induced by Atropine in Man

By *Joseph N. Berry, Howard K. Thompson, D. Edmond Miller and Henry D. McIntosh.* Department of Medicine, Duke Medical Center, Durham, North Carolina.

In spite of its increasing use as a method of inducing tachycardia, recent studies suggest that the circulatory effect of atropine is not limited to that of increasing heart rate. Cardiac output (dye-dilution), pulse rate, stroke volume, central blood volume, central venous pressure and total peripheral resistance were measured in 20 normal recumbent subjects before and after atropine (2 mg. i.v.).

Central venous pressure invariably fell and remained depressed during the study (mean fall 4 mm. Hg, $p < .001$). In observations from 30 to 60 seconds after atropine (4 subjects) cardiac output increased (62%) in proportion to the marked tachycardia (70%). Therefore, stroke volume did not fall significantly in the first minute following injection. However, in determinations made from 3 to 30 minutes following atropine, cardiac output (now increased only 28%) failed to keep pace with the persisting tachycardia (70%). This produced a consistent fall in stroke volume in all 20 subjects (mean fall 25%, $p < .001$). Mean arterial pressure increased (8 mm. Hg, $p = 0.01$), largely due to rise in diastolic pressure; peripheral resistance fell (18%, $p < .01$).

The atropine-induced reduction of stroke volume was not reversed by maneuvers intended to increase venous return or decrease venous pooling, i.e., elevation of the lower extremities, head-down tilt, anti-gravity suit inflation, or vigorous hyperventilation (normally effective in maintaining stroke volume despite marked tachycardia). In 4 subjects, however, rapid infusion of serum albumin (50 Gm.) did restore the reduced stroke volume to normal by effecting a striking additional increase in cardiac output.

The data clearly indicate that the effect of atropine is not limited to that of inducing tachycardia. The striking fall in venous pressure and stroke volume (after a short latent period) suggests that atropine induces venous pooling. These findings must be considered in future studies in which atropine is used as a means of inducing tachycardia.

### Hemodynamic Studies in Cardiac Tamponade

By *James K. Alexander and Edward W. Dennis.* Baylor University College of Medicine, Houston.

Five patients with pericardial effusion secondary to stab wound of the heart have been studied, 3 of whom had evidence of cardiac tamponade. At the time of right heart catheterization, cardiac output (Fick) was determined before and after pericardiocentesis. In addition, intracardiac, caval, pulmonary artery, intrapericardial, and intrathoracic (intraesophageal) pressures were measured.

In patients with tamponade, pericardiocentesis resulted in a rise in cardiac output due to increased stroke volume along with parallel decrements in right atrial and intrapericardial pressures. Associated increase in effective right ventricular filling pressure could not be clearly demonstrated. After pericardial tap, pulse pressure in the pulmonary artery rose considerably, while mean pulmonary arterial pressure fell somewhat. Little change occurred in systemic blood pressure after tap, so that the calculated systemic vascular resistance fell.

Little or no change was found in the gradient of pressure between the extrapericardial portions of superior or inferior vena cava and right atrium before and after tap, which has been taken as evidence against the concept that tamponade is associated with localized constriction of the great

veins as they pass through the pericardial sac. The diastolic dip in the right ventricular pressure curve seen with constrictive pericarditis is not present with pericardial effusion and cardiac tamponade. With tamponade, respiratory variations in intrathoracic pressure are not mediated to the intrapericardial or intracardiac chambers in normal fashion, being much less pronounced for a given change in intrathoracic pressure before tap than after.

**Quantitation of Valvular Regurgitation from Simultaneous Multiple Site Indicator Dilution Curves in Man**

By *Ramon L. Lange and Hans H. Hecht.* Department of Medicine, University of Utah College of Medicine, Salt Lake City. (Supported in part by a grant from the U. S. Public Health Service (National Heart Institute), and the Utah Heart Association.)

The estimation of regurgitation from arterial dilution curves suffers because the contour expected without regurgitation is unknown. The following procedure attempts separation of effects of flow (Q) and volume (V) from regurgitation (Qr).

Simultaneous dilution curves were obtained from the pulmonary artery (PA) and femoral artery (FA). After venous dye injection, blood was drawn through oximeters at a constant equal rate which minimized distortion by catheter passage. Transit time corrections were made.

In 15 nonregurgitant subjects, PA and FA curves were nearly identical despite variable heart size and flow. The product of Q and time between equivalent points on each curve yielded similar volumes. The difference of appearance times ($AT_{FA}$-$AT_{PA}$), and mean circulation times ($MCT_{FA}$-$MCT_{PA}$) in the nonregurgitant system yields $Q(\triangle AT) \cong Q(\triangle MCT)$.

In left heart regurgitation, the skewed FA curve is the composite of total flow: Qt. Qr, and net forward flow, Qf; the PA curve remains a function of only Qf. The shortest and average transit between PA and FA describe the QT and Qf respectively. Thus, Qt ($\triangle AT$) $\cong$ V; Qf ($\triangle MCT$) $\cong$ V. Recalling $Qr = (Qt - Qf)$, then $Qr/Qf \cong (\triangle MCT - \triangle AT)/\triangle AT$.

Model studies confirm the similarity of curves at two sites and known regurgitation was accurately predicted from altered dye curves. In patients with patent ductus arteriosus, RV and FA curves yielded "regurgitation" similar to the shunt determined by catheter sampling.

When left atrial sampling was substituted for the FA curve in mitral regurgitation (MR), the values of ($\triangle MCT - \triangle AT$) and $\triangle AT$ decreased proportionately, providing constant $\frac{Qr}{Qf}$ and an internal check on the method.

In 25 patients, this method predicted regurgitation as expected and found; it uncovered 4 unsuspected cases of MR, and only slight regurgitation in 4 patients previously considered inoperable because of erroneously suspected large insufficiency.

**An Experimental Evaluation of the Indicator-Dilution Technic for the Detection of Mitral Regurgitation**

By *Robert H. Eich, Ingolf Staib, Daniel M. Enerson and Henry Brown.* Department of Medicine and Surgery, State University of New York, Upstate Medical Center, Syracuse.

The indicator-dilution technic has been widely applied in the detection of mitral regurgitation. However, much of the work has been done in patients where precise evaluation of the mitral valve lesion was often not possible. It was felt therefore to be of value to evaluate the technic in an experimental preparation where the regurgitant flow could be controlled and measured.

Regurgitation was produced in the open-chested dog by an external polyvinyl shunt between the left ventricle and left atrium. Regurgitant flow was measured through a side arm and reservoir set at left auricular level. Fifteen dogs were studied. Cardiac output was measured using radioactive iodinated human serum albumin as the indicator. Injections were made into the left auricle or pulmonary artery, and interrupted samples at one-second intervals taken from the femoral artery. Regurgitant flow varied between 20 and 38% of forward flow (mean 27%).

There was no significant difference between the forward output with or without the shunt being open. There was a good correlation between cardiac output and central blood volume ($r = .87$) but none between slope and cardiac output. In the same dog, the curve obtained with the shunt open could always be separated from the control curve with the shunt closed by using any simple analysis such as slope or disappearance time. However, when the data from the 15 dogs were pooled, none of the currently used analysis involving slope, time and/or concentra-

tion ratios would completely separate the control group from that with insufficiency.

Using the interrupted sample method for obtaining dye dilution curves in this experimental preparation, it can be said that given a control dye curve for comparison, the method is sensitive enough to detect 20–38% regurgitation. However, with one isolated measurement the presence of mitral regurgitation cannot always be determined.

### Some Factors Affecting Indicator Dilution Curves in the Presence and Absence of Valvular Incompetence

By *J. I. E. Hoffman and George G. Rowe.* Department of Medicine, Postgraduate Medical School, Hammersmith Hospital, London.

Dye dilution curves were made in a circulation model in which the amount of forward and backward flow past an incompetent valve could be measured directly. First it was confirmed that the slope, variance, appearance time, mean curve time and curve duration were, in the absence of valvular incompetence, determined chiefly by forward output and central volume and only slightly by large changes of stroke and residual volume of the pump. The "atrio-ventricular" valve was then made incompetent and changes were made in the size, shape and elasticity of the proximal chamber entered by the regurgitant jet. With a narrow rigid proximal chamber there was little distortion of the curve despite backflows of about 75% of the forward flows; large rigid chambers allowed slightly more curve distortion which was barely detectable. When, however, a large elastic proximal chamber was used gross distortion of the dye curve occurred, the amount of distortion depending not only on the amount of backflow but also on the size of the proximal chamber. The type of change in the curve was that described by Korner and Shillingford (i.e. earlier appearance time, longer and lower curve), but the backflow estimated in our model by their formulae could differ greatly from the measured backflow and depended greatly on the type of proximal chamber.

The hypothesis was therefore made that the distortion of the indicator dilution curves produced by backflow is due to the dilution and subsequent washout of regurgitated indicator, and that this depends not only on the amount of backflow but also on the nature of the regurgitant jet and the chamber which it enters. Many of the discrepancies found in practice in attempting to measure valvular regurgitation by indicator dilution methods may be accounted for by this hypothesis.

### Left Heart Catheterization

By *Julius E. Birnbaum, Harold Selinger and Irving G. Kroop.* Cardiopulmonary Units of the Jewish Hospital and the Jewish Chronic Disease Hospital, and Department of Medicine, State University College of Medicine, Brooklyn, New York. (Aided by the Udo Reinach Fund and the Mildred Forman Foundation.)

The indications and limitations of left heart catheterization by the posterior thoracic approach are based on left atrial, left ventricular, aortic, central and peripheral arterial pulse contours in 40 selected patients.

The technic has been helpful in determining the presence or absence of a significant systolic ventricular-aortic gradient in aortic insufficiency, aortic stenosis and in combined aortic valvular lesions. Assigning the cause of the anginal syndrome to either aortic stenosis or coronary sclerosis has been possible. Persistent mitral stenosis after commissurotomy can be diagnosed by the presence of a diastolic left atrioventricular gradient. Characteristic left atrial contours have been obtained in "pure" mitral insufficiency and in predominant mitral stenosis.

Left heart catheterization established the diagnosis of mitral insufficiency in a patient with right-sided pressure-pulse contours simulating constrictive pericarditis. Normal pressures and pulse contours have ruled out valvular lesions in idiopathic cardiac hypertrophy.

Left heart catheterization is limited frequently by the inability of directing the plastic catheter into the desired chamber particularly in the presence of severe regurgitation or stenosis. Damping of the pressure-pulse contours may reduce their diagnostic value.

The left atrial tracing alone has limited value in the diagnosis of the degree of mitral insufficiency in the combined mitral valve lesion, particularly when atrial fibrillation and heart failure are present.

### Appraisal of Left Heart Catheterization and Dye Dilution Technics in the Diagnosis of Mitral Valve Disease

By *R. W. Gunton, W. Paul and R. O. Heinbecker.* University of Toronto, and Cardiovascular Unit, Toronto General Hospital.

Experience with 180 left heart catheterizations (Bjorck) in problem cases of valvular heart disease has prompted assessment of pressure pulse in left atrium (LA), diastolic atrioventricular gradient, and dye dilution technics in distinguishing dominant stenosis and insufficiency of the mitral valve.

Analysis of LA pulse contour in 50 patients with valve disease of proved type (19 pure stenosis, 18 significant stenosis with moderate insufficiency, 13 pure insufficiency) showed: (1) the rate of *y* descent of the *v* wave divided by the height of the *v* wave (Ry/v, Owen and Wood) did not distinguish stenosis from insufficiency; (2) *y* descent during the first .10 sec. divided by mean left atrial pressure (MLAP) (Morrow et al.) was more decisive; (3) the best indication of pure stenosis was *v* not exceeding *c* by more than 3–4 mm. in the presence of elevated MLAP; (4) no method was without error.

In proven stenosis the mid-diastolic gradient between atrium and ventricle was usually 15–20 mm. Hg, rarely 30 mm., and in cases with low flow 6–8 mm. A gradient did not prove stenosis; 2 patients with early diastolic gradients of 11–15 mm. were found to have "pure" insufficiency with immobile cusps.

In 20 patients estimates of regurgitant flow calculated by the Korner–Shillingford dye curve method agreed only qualitatively with the known valve lesion.

The most direct estimate of presence and degree of mitral insufficiency was achieved in 14 patients by injecting T-1824 into left ventricle and sampling by cuvette oximeter from LA. Area of "regurgitant flow" so obtained was compared with net forward flow determined simultaneously by ear oximeter curve or by a second injection into right atrium, with sampling from LA or ear oximeter. In 6 patients a repeat ventricular injection was made after moving the tip of the LA needle 2–3 cm. The ratio of regurgitant to forward flow curve areas was unchanged, providing no evidence for incomplete mixing of dye in LA.

### Use of Intravascular Carbon Dioxide to Demonstrate Interatrial Septal Defects

By *William L. Winters, Jr., Michael Wilson, Herbert M. Stauffer and M. J. Oppenheimer.* Departments of Medicine, Radiology and Physiology, Temple University Medical Center, Philadelphia.

In previous studies the intravascular injection of carbon dioxide has been shown to be a safe tool for investigation of intracardiac structure and function. The present study was designed to study the cardiovascular effects of carbon dioxide in dogs in the presence of experimentally produced atrial septal defects and to determine if detection of such defects could be made roentgenographically following the intravascular injection of carbon dioxide. The problem of residual gas bubbles was also to be investigated.

Atrial septal defects were produced by the method of Blalock and Hanlon in mongrel dogs anesthetized with pentobarbital. The dogs were placed in various positions between an x-ray source and a fluoroscopic image intensifier to which a 16 mm. Bell & Howell camera was attached. Carbon dioxide was injected in various venous and intracardiac locations. Records of electrocardiogram, intracardiac and peripheral pressures, and respirations were made simultaneously with the moving picture obtained. Oxygen studies, dye dilution curves with Evans Blue and autopsy findings were obtained to confirm the presence of atrial septal defects.

*Results:* Atrial septal defects measuring 0.5 to 3.0 cm. in diameter were produced in every dog. The defect was detected by 2 x-ray criteria regardless of direction of shunt as determined by oxygen studies. Gas was seen briefly in the left atrium. An air fluid level was visible in *each* ventricle for 10–15 seconds. The defect was demonstrable by other means. Intravenous injection of carbon dioxide in dogs with intact septums produced transient profound drop in left ventricular and femoral arterial pressures. In the presence of the defect there was, in contrast, a rise in these pressures. No fatalities occurred following escape into pulmonary artery or systemic circulation of residual bubbles trapped for up to 2 minutes from the left or right ventricle.

*Conclusion:* The intravascular injection of carbon dioxide has been shown to be a safe and useful method to detect the presence of interatrial septal defects in anesthetized dogs by two roentgenographic criteria and by characteristic pressure changes under the conditions described. Residual gas bubbles have posed no problem.

### Congenital Bicuspid Aortic Valves: A Villain in Aortic Stenosis Too?

By *Michael B. Matthews and Anthony Bacon.* Department of Medicine, University of Edinburgh and St. Thomas' Hospital, London. (Aided by a research grant from the Scottish Hospitals Endowment Research Trust.)

It has been shown that in association with coarctation of the aorta congenital bicuspid aortic valves are liable to develop aortic stenosis. This study was made in order to determine whether this was also true in a wider context.

Specimens of bicuspid aortic valves were examined from 8 of the major London pathological museums. If there was a history of rheumatic fever, an abnormality of the mitral valve, or if it appeared that the valve was bicuspid because 2 of 3 equal cusps had fused, the specimen was excluded.

There were 28 specimens for which the sex and age at death were known. In 24 of these the cusps were of equal size; in the other 4 specimens one cusp was slightly larger than the other and they were included because there was an associated congenital anomaly.

Twenty-two of the 28 were male. Nine had a co-existing congenital abnormality. Thickening of the valve cusps increased with age. Calcification of the cusps appeared in the 4th decade, and was found in all of the 9 specimens aged more than 60 at the time of death. A central radial ridge was present in one cusp in 6 specimens; in 2 of these there was a co-existing anomaly (coarctation and bicuspid pulmonary valve). Seven specimens had bacterial endocarditis, and 14 aortic stenosis.

When there was evidence of obstruction to blood flow at the aortic valve from left ventricular hypertrophy or from the clinical picture, it was more often due to valve rigidity than to fusion of the 2 cusps.

It is suggested that bicuspid aortic valves may be as liable to develop aortic stenosis in old age as subacute bacterial endocarditis at an earlier period. In this way they may contribute to the elderly cases of aortic stenosis, as suggested by Peacock 100 years ago.

### Osteitis Deformans and Calcific Disease of the Heart Valves

By *Herbert Hultgren and Edward Caul.* Department of Medicine, Stanford University School of Medicine, San Francisco. (Aided by a grant from the San Francisco Heart Association.)

Osteitis deformans (Paget's disease of bone) has been alleged to involve the cardiovascular system by an increased incidence of calcific valvular disease and an excessive degree of arteriosclerosis. Observations supporting these contentions, however, are scanty. For this reason a study has been made of 88 patients with severe, generalized osteitis deformans and 618 control patients in a similar age group who were not affected by the disease. Data were obtained from clinical records, autopsy protocols, and, in 50 control patients, by dissection and direct study of valvular calcifications. The following observations were made: in 27 autopsied cases of severe osteitis deformans, grossly visible calcification of the mitral and aortic valves was present in 59% and severe aortic stenosis was found in 24%, compared to an incidence of 18% and 3.5% respectively in 201 control cases. Clinically evident aortic stenosis was present in 21% of 66 patients with osteitis deformans, compared to an incidence of 4.3% in 417 control patients.

The severity of arteriosclerosis of the coronary arteries, aorta, and pelvic vessels was similar in the 2 groups.

Despite recent studies indicating that some patients with osteitis deformans may have an elevated cardiac output, an analysis of blood pressure, heart rate, pulse pressure and heart weight from patients with severe osteitis deformans revealed no deviation from similar data obtained from control subjects.

It is therefore concluded that an important effect of severe osteitis deformans upon the cardiovascular system is the development of an increased incidence of calcific valvular disease of both mitral and aortic valves, resulting in a striking increase in the incidence of clinically significant aortic stenosis. In addition, these data provide further support to the concept that aortic stenosis in the elderly may be of nonrheumatic origin.

### The Prognosis of Rheumatic Carditis

By *Alvan R. Feinstein and Rodolfo Di Massa.* Department of Medicine, New York University College of Medicine, Irvington House, Irvington-on-Hudson, New York.

The present study was done to learn which clinical features are most significant in predicting the outcome of an attack of rheumatic carditis. The patients were 315 children and adolescents observed at monthly intervals at the Irvington House Prophylaxis Study Clinic following an initial attack of unequivocal rheumatic fever, without a recurrence. The follow-up period has averaged 4.8 years (range 2–9 yrs.) and 78% of the patients have been followed for 4 years or more.

The patients were classified according to the murmurs present during the acute attack, regardless of whatever other features of "carditis"

may have occurred. The designation "valvulitis" was given to 96 patients, without previous heart disease, in whom a definite diastolic murmur appeared or disappeared during the acute attack. Of these, 63 (66%) now have rheumatic heart disease. The designation "probable valvulitis" was given to 52 patients who developed (1) a new loud apical systolic murmur radiating to the axilla but no diastolic murmur or (2) a questionable diastolic murmur. Of the patients in this group, 15 (29%) now have heart disease.

The designation "no valvulitis" was given to 167 patients who did not meet the above criteria for valvular involvement, although most of these patients had other systolic murmurs and many had prolonged P-R intervals, gallop rhythm, "changes" in cardiac size and other features of rheumatic "carditis." Of these patients, none has rheumatic heart disease today.

Many more years of further observation will be needed to confirm these results. However, the existing data strongly suggest that the auscultatory phenomena present during the acute attack of rheumatic fever are of primary importance in predicting the cardiac prognosis. Patients without "valvulitis" or "probable valvulitis" may be reasonably assured that subsequent heart disease will not develop in the absence of rheumatic recurrences. The results also indicate renewed importance for the stethoscope as a diagnostic tool.

### Plasmin Lysis of Experimental Coronary Thrombi

By *Paul Ruegsegger, Irwin Nydick, Alvin Freiman, Nils Bang, Steven Kanor, Eugene E. Cliffton and John S. LaDue.* Sloan-Kettering Division of Cornell University Medical College, New York.

The effects of the fibrinolytic enzyme plasmin upon experimental coronary thrombi and the myocardium were studied in open-chest dogs by direct coronary arteriography and histologic examination.

The minute anatomy of the coronary vessels was delineated by means of serial arteriograms by the injection of Hypaque through a #190 polyethylene catheter inserted into a small proximal side branch of the major vessel under study during control periods and after coronary blockade.

The coronary thrombi were produced by inducing a "hypercoagulable state" in a distal isolated segment of a major artery by a modification of the method previously described by Sanford Wessler. Intracoronary clotting occurred within 2 minutes following the injection of a mixture of blood and serum from the operated animals.

The arteriograms accurately demonstrated the clots as well as the resulting degree of obstruction in the control and treated groups. In 5 control experiments, the clots were relatively unchanged for 10 to 12 hours. Clot retraction of approximately 30% was noted in 2 animals.

Complete or partial lysis of coronary thrombi within 1 to 6 hours, resulting in restoration of blood supply to the ischemic myocardium, was achieved in 100% of plasmin-treated animals. These 7 animals received 4000 plasmin units/Kg./hr. by systemic administration resulting in significant fibrinolytic activity.

Gross and microscopic examination of animals autopsied within 12 hours after occlusion revealed striking differences between the 2 groups. The untreated hearts in addition to the primary thrombus showed marked capillary engorgement with frequent thrombi and interstitial edema of the infarcted and marginal ischemic zones, whereas the plasmin-treated hearts showed edema only. The late effects of these observed changes upon muscle cell damage are under study. There was no pathologic evidence that plasmin caused hemorrhage or any other injury to the heart structures.

### Increased Survival From Use of Central Nervous System Depressants in Acute Coronary Occlusion in the Dog

By *William Regelson, F. Stanley Hoffmeister and Heino Rubin.* Roswell Park Memorial Institute, Buffalo.

Survival following acute coronary occlusion in the dog has been studied under varying conditions of tranquilization, analgesia and anesthesia.

Loose ligatures were placed around the left anterior descending coronary at its junction with the circumflex artery and the free ends brought out through the chest wall. At least one week later when the dogs were fully active, sudden occlusion was produced by drawing the ligature ends tight. Serial EKG's and blood pressure tracings were obtained. Heart specimens, verifying occlusion, were obtained in all experiments.

The blood pressure fall following occlusion is associated with the onset of ventricular fibrillation or ventricular tachycardia which leads to

fibrillation. All animals who experienced ventricular fibrillation died, and all survived in whom ventricular fibrillation did not appear. If dogs did not die within 15 minutes they usually survived indefinitely, and recovery was defined as survival beyond 1 hour following occlusion. Myocardial infarction was evident in all survivors; congestive failure occurred in only one out of 24.

In 6 of 7 untreated conscious dogs death occurred within 5 minutes following occlusion. When 25 μg./Kg. of reserpine was administered i.m. 150 minutes prior to occlusion, 8 of 10 dogs recovered. Pentobarbital anesthesia (25 mg./Kg.) was associated with survival in 4 of 4 dogs. Four animals received morphine sulfate 30 minutes before occlusion and all recovered.

When chlorpromazine (2 mg./Kg.) was given to conscious dogs i.v. immediately *after* occlusion, 7 of 10 recovered.

Central nervous system depressants (reserpine, pentobarbital, morphine sulfate, chlorpromazine) significantly increase survival following acute coronary occlusion in the dog, and this is apparently mediated through suppression of ventricular fibrillation.

### Comparison of SGO-T, SGP-T and SL-D Activity in Acute Myocardial Infarction and Acute Myocardial Ischemia

By *Nils Bang, Paul Ruegsegger, Irwin Nydick and John S. LaDue.* Sloan-Kettering Division of Cornell University Medical College, New York.

The activities of serum glutamic oxaloacetic transaminase (SGO-T), serum glutamic pyruvic transaminase (SGP-T) and serum lactic dehydrogenase (SL-D) were measured daily for 6 to 11 days in 31 patients with unequivocal acute transmural myocardial infarction, in 31 patients with coronary insufficiency with and without evidence of acute infarction, in 3 patients with acute pulmonary infarction and in 3 during and following acute hemorrhagic shock. An additional 6 patients with congestive heart failure and 8 with arteriosclerotic heart disease were similarly studied.

In the 31 patients with acute infarction the peak SGO-T activity was 3 to 4 times normal in 29, the SGP-T twice normal in 27 and the SL-D activity 2½ times normal in 26. The SGO-T activity returned to normal within 5 days, the SGP-T in 6 days, and the SL-D in 6 to 11 days. The maximum activity after infarction was usually within 24 hours for SGO-T and SGP-T, but took 48 hours for SL-D.

The SGO-T when measured within 3 days of the onset of acute myocardial infarction was more consistently increased than either SGP-T or SL-D. SL-D activity remained elevated for 2 to 5 days longer than did SGO-T or SGP-T.

A rough correlation was found between the severity of the clinical condition and the maximum levels of all 3 enzymes. Patients who died 3 days to 3 weeks after the myocardial infarction showed peak activities 50 to 100% higher than the group of patients who survived. The patients who were in shock for a shorter or longer period of time showed higher peak activities of all 3 enzymes than the patients who were not in shock.

Of 30 patients who presented the electrocardiographic picture of coronary insufficiency, 15 were diagnosed as sustaining myocardial infarction and 15 patients as cases of coronary insufficiency without infarction on the basis of enzyme determinations. The best positive correlation between the clinical and "enzyme" diagnoses was seen to exist for SGO-T; the negative correlations for the 3 enzymes were of the same order of magnitude.

In a control group of patients with a variety of cardiovascular diseases, minimal elevations of SGP-T and SL-D were found in 7 while the SGO-T was slightly increased in 5 patients.

### The Unreliability of Subjective Circulation Time Determinations

By *Murray M. Mahl and Kurt Lange.* Vascular Research Group, New York Medical College-Metropolitan Medical Center (Bird S. Coler Hospital). (Aided by a research fellowship grant of the U.S. Public Health Service.)

Since the determination of circulation time is highly important for the evaluation of circulatory efficiency, the fully objective dermofluorographic method of measuring circulation time was compared with the more generally used subjective decholin and saccharin methods to evaluate their accuracy.

The subjective methods depend upon the reaction time of the patient and the physician and may vary in the same individual under identical conditions, and are associated with a large percentage of "blanks." They are often dangerous, and cannot be performed in unconscious patients and in small children.

The dermofluorographic circulation time is

a completely objective graphic automatic method of recording circulation time using fluorescein. The time elapsing from the intravenous injection of 3 ml. of 5% fluorescein solution to its arrival in the skin of the face is automatically recorded photoelectrically. The instrument is capable of ascertaining a fluorescein concentration of 1 part in 30 million. The method is repeatable and its accuracy lies within ± 5%; it may be performed on unconscious patients, infants and children, and surmounts all language barriers. The instrument is portable and the test is performed at the bedside.

In 100 cases, 62 of which suffered from heart disease in various stages, a comparative study of the circulation time determined with decholin and the dermofluorographic method carried out simultaneously revealed a large unpredictable error of the decholin method with a wide scattering of the variation in the normals and especially in the cardiacs in failure.

In 34 cases, a comparison study of the saccharine and dermofluorographic circulation time showed that the saccharine time was still less reliable than the decholin time, with an even greater unpredictable error with still wider scattering. It appears that large residual cardiac volumes tend to increase the error of the subjective methods.

### Influence of Ouabain on the Hypothermic Canine Heart

By *E. T. Angelakos.* Department of Physiology, Boston University School of Medicine, Boston.

The purpose of the experiments described herein was to determine whether digitalization altered in any way the well known susceptibility of the hypothermic heart to ventricular fibrillation (VF).

In a series of 30 animals the incidence of spontaneous VF following progressive hypothermia was determined in digitalized and non-digitalized groups with and without atropine treatment. The results indicate that the small differences existing between control and digitalized groups could be attributed to the cholinergic actions of ouabain since they were absent in the atropine-treated groups.

The toxicity of ouabain was determined in a series of 20 dogs. In normothermic animals doses of 0.02 mg./Kg. produced ventricular extrasystoles within 15 to 30 minutes following injection. By contrast in hypothermic animals (heart temperature 26 ± 1 C.) ouabain in doses of 0.1 mg./Kg. did not produce extrasystoles up to 2 hours after injection. Extrasystoles and VF occurred in hypothermic animals following doses of 0.15 to 0.20 mg./Kg.

In a third series of 20 animals right ventriculotomy was performed, in control and digitalized (0.02 mg./Kg.) groups, during a 15-minute period of inflow occlusion at heart temperatures of 26 ± 1 C. The incidence of operative VF in the two groups was essentially identical.

The over-all results indicate that digitalization does not alter in any way the susceptibility of the hypothermic heart to VF. Furthermore the data show that the toxicity of ouabain in producing ventricular extrasystoles is decreased substantially by hypothermia.

### The Effect of Ganglionic Blockade on Venous Pressure and Blood Volume: Further Evidence in Favor of Increased Venomotor Tone in Congestive Heart Failure

By *David H. Lewis, Manuel Cardenas and Herschel Sandberg.* Division of Cardiology, Philadelphia General Hospital, Philadelphia. (Aided by a grant from the National Heart Institute of the National Institutes of Health.)

Previous work by other investigators has demonstrated that ganglionic blockade lowers venous pressure in patients with congestive heart failure (CHF). These observations, interpreted as demonstrating increased venomotor tone in CHF, have cast doubt on the "classical" concept of increased blood volume as the determinant of venous hypertension. These 2 hypotheses are not contradictory if the lowering of venous pressure by ganglionic blockade is due to alterations in blood volume. The purpose of this study was to rule out changes in blood volume as the factor responsible for the drop in venous pressure.

Forty patients were studied. Twenty had moderately severe to severe CHF. Twenty, who served as controls, were for the most part without heart disease or, if heart disease could not be ruled out, were never in CHF. After 1 hour at rest, hematocrit was drawn and 15 μc. of radioactive iodinated serum albumin (RISA) was given. Samples were drawn at 10, 15, and 20 minutes. Following this, 10 normals and 10 with CHF were given hexamethonium (2.5 mg./min. for 20 min., total dose 50 mg.). The remainder were given 5% dextrose. The blood volume was then repeated in the same manner using 90 μc. of RISA. Arterial pressure (auscultatory),

pulse rate, and venous pressure (electromanometer) were measured at frequent intervals.

In CHF hexamethonium caused a precipitous and persistent drop in venous pressure. The blood volume remained essentially unchanged. Normals given hexamethonium showed a variable venous pressure drop and no change in the blood volume. Normals and CHF without hexamethonium showed no change in either venous pressure or blood volume.

It has been demonstrated, therefore, that in CHF a strict dependence of venous pressure on blood volume does not exist. This is interpreted as further evidence that increased venomotor tone is most likely the important factor in the genesis of venous hypertension in CHF.

**Postural Potentiation of Diuresis in Intractable Congestive Heart Failure: Circulatory and Volume Factors**

By *Marian C. Isaacs, Jacob Grossman, Robert Rosenblum and Raymond E. Weston.* Medical Division, Montefiore Hospital, New York.

Elevation and elastic bandaging of the lower extremities markedly enhances mercurial diuresis in patients in severe congestive failure. To investigate the factors underlying this augmented response, the following parameters were measured in 7 cardiac patients prior to and during elevation and bandaging of the legs: antecubital, iliac and central venous pressures, plasma volume, hematocrit, renal hemodynamics, urine flow and electrolyte excretion. In these patients, on leg elevation, the initially high antecubital venous pressures exhibited a sustained increase of 3–6 cm. water unlike the smaller (2 cm. water) transient rise encountered in normal subjects. In 2 patients, the right iliac venous pressures paralleled the antecubital both before and during leg elevation. In cardiacs, the transmitted central venous pulse contour tended to disappear during early diuresis, but recurred following leg elevation, suggesting its relationship to venous distention. In 2 patients, right atrial pressure was unchanged by leg elevation.

The plasma volume (T-1824 or I131 RISA), initially high, was unchanged or decreased slightly during early diuresis. Following leg elevation, although no significant increase could be demonstrated, the plasma volume remained constant despite prolonged marked diuresis. The hematocrit remained unchanged, providing added support for the belief that the plasma need only maintain its volume in order to provide the necessary stimuli for diuresis. The failure to demonstrate measurable increases in plasma volume is attributable to the kidney's ability to remove fluid at a rate equal to or greater than its transport to the vascular compartment.

In 5 patients, leg elevation produced a 12–25% increase in GFR and 6–16% increase in RPF; these changes persisted throughout the duration of elevation. Coincidentally, significant increases in urine flow and natriuresis were observed. The above findings suggest that maintenance of effective circulating volume is essential to the continuance of diuresis.

**The Relation of Emotional State to Renal Excretion of Fluids and Electrolytes in Patients with Congestive Heart Failure**

By *Robert Barnes and William Schottstaedt.* Department of Medicine, University of Oklahoma, and Medical Service, V. A. Hospital, Oklahoma City. (Aided by a grant from the National Heart Institute, USPH.)

Earlier studies have demonstrated a correlation between variations in renal excretion of sodium and water and the emotional state in healthy individuals. The present study was undertaken under more precisely controlled circumstance in an effort to define the degree of change which may occur and to determine its potential importance in the management of patients with congestive heart failure.

Eight male patients with congestive heart failure, but stabilized as to weight, were studied on a metabolic ward for periods ranging from 14 to 45 days. Sodium and water intake were maintained constant. All urine was collected and appropriately analyzed keeping the data separate from a daily appraisal of the behavior, attitudes and emotional state of the subject. The contribution of body position and muscular activity to the excretory pattern were also controlled and evaluated. There were 685 separate specimens analyzed.

It was found that in states of discouragement or tension, excretion of sodium fell to as low as 12% of control value and water as low as 35%. Renal excretion of sodium ranged up to 400% of control values during relaxation following tension or depression. In states of apprehension or anger similar increase in urinary sodium was found. In all these emotional states fluctuation in potassium excretion was minimal. The alterations in excretory pattern attributable to body position or muscular activity were minor in comparison.

## Magnified Electrocardiograms

By *George A. Kelser, Jr. and Cesar A. Caceres.* Department of Medicine, George Washington University School of Medicine.

Electrocardiographic interpretation is best learned by reading a large number of electrocardiograms under the tutelage of an experienced cardiologist. The conventionally recorded electrocardiogram is too small to permit close and simultaneous examination by more than one or two individuals. A larger tracing would permit the instruction of more trainees with better utilization of the instructor's time.

The smallness of the waves and the time markings of the electrocardiogram result in unavoidable visual errors in routine mensuration. Such errors are particularly noticeable when a great number of electrocardiograms must be read by one individual. Some tracings of low voltage cannot be interpreted simply because visualization of the waves is inadequate.

The smallness of the usual trace is also a detriment to its use in circumstances other than routine clinical interpretation and teaching. Planimetry of wave areas is impossible without magnification. The precise location of instantaneous vectors of less than 0.04 seconds, or, at times, the definition of onset and end of a wave are difficult to determine without magnification.

A magnifying device has been developed that provides: (1) visualization of at least 3 cardiac cycles from either photographic or heat-sensitive records; (2) a parallax-free image with a magnification factor of 10 to permit conversion of time, magnitude or area values easily; (3) a rate tabulation mechanism and moveable pointers for vector symbols; and (4) daylight viewing.

The device is of value in routine clinical interpretation of electrocardiograms and in teaching electrocardiography.

## The Normal P Wave

By *Cesar A. Caceres and George A. Kelser, Jr.* Department of Medicine, George Washington University School of Medicine.

The precise duration of the P wave is important in correlative studies in electrocardiography, in the use of the electrocardiogram to time physiologic events such as heart sounds and vascular pressure curves, and in the measurement of the PR interval. Variability of the normal range quoted in the literature limits the clinical application of this measurement. The present study was performed to determine if it were possible to obtain better definition of the duration of the P wave.

The P wave was studied in 50 electrocardiograms of normal male subjects by means of an electrocardiographic enlarger that magnified the tracings 10 times and thereby allowed more precise measurement.

The distribution of the values of the durations of the six hundred P waves ranged from zero to 0.178 seconds. The average P wave duration was 0.101 seconds. The average maximum duration was 0.130 seconds. Forty-four of the 50 subjects had a P wave of 0.12 seconds or greater in one or more leads.

Average values obtained for the P wave duration in all leads and the average value of the P wave duration in each of the 12 leads of the routine electrocardiogram did not closely represent the range of the P wave duration in this group. The lead which demonstrated the longest P wave duration was not predictable. The most reliable measurement for a given subject would appear to be the maximal duration encountered in his electrocardiogram. Random selection of a lead for measurement of P wave duration to time physiologic events may introduce significant error.

The electrocardiographic literature states that P waves exceeding 0.12 seconds or more are abnormal. Since the values of the P wave duration in this group of normal subjects were often greater than 0.12 seconds, a review of the present criteria for normal P wave duration is needed.

## Evaluation of Quantitative Methods in the Interpretation of the Electrocardiogram and Vectorcardiogram

By *Hubert V. Pipberger.* Cardiovascular Research Laboratory, Georgetown University Hospital, Washington, D. C. (Aided by a grant of the American Heart Association.)

Quantitative methods for the analysis of the ECG and VCG have been proposed in order to obtain better separations between normal and abnormal. Results of such studies have not been encouraging. Therefore, the reliability of some commonly used analytic criteria was investigated in a group of 100 normal subjects. Schmitt's corrected SVEC III leads were used for simultaneous scalar ECG and three-planar VCG recordings.

A simultaneous onset of QRS in scalar leads was found in only 29% of the cases. The mean time discrepancy for the onset was 0.015 ± 0.005 sec. Accurate determinations of initial vectors from singly recorded leads cannot be made.

In 29% of the cases initial QRS forces were perpendicular to one of the VCG planes, adding to difficulties in measurements around point E.

The direction of 0.01 sec. QRS vectors in the frontal plane was a function of the direction of their maximal QRS vectors. Grouping according to the direction of the latter vectors led to significant differences between 0.01 sec. QRS directions ($P < 0.001$). This interrelationship has to be taken into account quantitatively in Q-wave and initial vector analysis.

Peak deflections of scalar leads showed a mean time discrepancy of 0.014 ± 0.005 sec., precluding their use in plotting "mean" QRS vectors. Due to this discrepancy maximal QRS vectors were identical in all planes in only 10%. The mean spatial angle between maximal vectors of the frontal and horizontal planes was 34 ± 33.9°. Maximal (often erroneously called "mean") QRS vectors can therefore not be used for quantitative spatial analysis. Such procedures require an identical vector for all planes.

It appeared from the results that a thorough re-evaluation of analytic methods is needed in order to obtain more reliable means for quantitative ECG evaluations. Past discouraging results can be attributed to use of faulty methods.

### An Unusual Normal Electrocardiographic Variant

By *James P. Diestel, Jr. and George H. Reifenstein.* Medical Service, St. Mary's Hospital, San Francisco, and Cardiology Department, U.S. Naval Hospital, Oakland, California.

The purpose of this study is to direct attention to an unusual normal ECG variant.

During a 30-month period approximately 15,000 consecutive ECG's were analyzed for this pattern. The routine 12 leads were used. Six instances were found, all in males, of whom 5 were Negroes; the age range was 19–31 years.

The pattern was characterized by (1) RST elevation in limb and various precordial leads, (2) variable RST depression in $aV_R$, (3) T-wave incisions, $V_3$-$V_6$, (4) unchanging patterns over months or years, (5) no changes following hyperventilation, electrolytes, anoxia, exercise, smoking, various drugs, or alterations in position or breathing.

We conclude that although these changes mimic pericarditis or myocardial diseases, a consideration of clinical data, the above features, and a rather distinctive low junctional take-off of the RST elevation serves to distinguish the pattern from pathologic conditions.

### Reversal of the Cardiotoxic Effects of Quinidine by Molar Sodium Lactate

By *Samuel Bellet, Guillermo Hamden and Andrew Somlyo.* Division of Cardiology, Philadelphia General Hospital, Philadelphia. (Aided by a grant from the National Institute of Health.)

The administration of molar sodium lactate to a patient resulted in a reversal of the cardiotoxic effects with quinidine toxicity. Because of the beneficial effects of molar sodium lactate in hyperkalemia and the similarity of the electrocardiographic effects of quinidine toxicity, the following studies were performed:

Quinidine gluconate was administered to 5 dogs at a rate of 1/mg./Kg./min. and the following studies were obtained: continuous electrocardiogram and blood pressure recording, quinidine levels, pH and serum electrolytes (potassium, sodium, magnesium, calcium and chloride). These studies showed a gradual and constant progression in the severity of the electrocardiographic findings and a fall of blood pressure as the quinidine levels increased. Death of the animal occurred with a slow idioventricular rhythm.

These studies were repeated in 10 dogs following the administration of molar sodium lactate during various stages of quinidine cardiotoxicity. Following the infusion of molar sodium lactate there resulted an almost immediate reversal of the cardiotoxic effects as manifested by a decrease in QRS widening and an increase in blood pressure. This was accompanied by a decrease in the quinidine plasma concentration, increase in pH, decrease in serum potassium and an increase in serum sodium. Similar results were obtained 2 or 3 times in the same animal.

The following are suggested theories for the cause of the improvement: (1) decrease in plasma quinidine concentration; (2) decrease in serum potassium concentration. There is some evidence that potassium and quinidine may manifest synergistic effects on the heart. These findings may have a bearing on the clinical problem of treating quinidine toxicity.

### A Method for the Treatment of Quinidine and Procaine Amide Intoxication: A Clinical and Experimental Study

By *Fred Wasserman, Leonard Brodsky, John H. Kathe, Paul L. Rodensky, Morris M. Dick and Peyton S. Denton.* Departments of Cardiology and Medicine, University of Miami Medical School, and V. A. Hospital, Coral Gables, Florida.

Molar sodium lactate has been shown to narrow widened QRS complexes and to increase depressed cardiac rhythmicity due to various etiologies. The purpose of this paper is to report our observations on the effects of molar sodium lactate in experimental quinidine and procaine amide intoxication, and to demonstrate the successful application of these findings in accidental drug intoxication in man.

Forty lightly anesthetized mongrel dogs were studied at varying stages of toxicity with quinidine and procaine amide. Continuous arterial pressures and electrocardiograms were recorded; serial arterial blood samples were obtained to study changes in pH, serum electrolytes and chemistries. Injectable quinidine hydrochloride was administered intravenously at a rate of 3.4 to 5.7 mg./Kg./min. (20 dogs); procaine amide was infused at a rate of 100 to 150 mg./min. (20 dogs). At a desired stage of intoxication the quinidine or procaine amide infusion was stopped and intravenous sodium lactate was started at a rate of 3 to 10 cc./min. Control animals were not given molar sodium lactate.

Sodium lactate rapidly narrowed the widened ventricular complexes resulting from quinidine and procaine amide intoxication, promptly restoring conduction disturbances and arrhythmias to normal; the acidosis that uniformly resulted from the intravenous administration of quinidine hydrochloride was corrected; hypotension accompanying both procaine amide and quinidine intoxication was returned to normal levels. The administration of molar sodium lactate prior to giving quinidine or procaine amide appears to significantly increase the tolerance of the experimental animal to both drugs.

Experience with patients demonstrated effectiveness of molar sodium lactate in the treatment of severe quinidine intoxication (QRS complexes widened to 0.20 sec. with A-V dissociation). Our preliminary impression is that sodium lactate may reverse these toxic effects of quinidine and procaine amide without changing the therapeutic effects.

### The Relationship of Blood Pressure to Changes in Body Fluid and Electrolytes in Steroid Hypertension

By *Aram V. Chobanian, Belton A. Burrows and William Hollander.* Robert Dawson Evans Memorial Hospital, Massachusetts Memorial Hospitals, Boston.

Changes in body fluid and electrolytes were studied in subjects with "steroid hypertension" utilizing metabolic balance and radioisotope dilution technics.

All subjects with primary hyperaldosteronism and Cushing's Disease had significant reductions in exchangeable body potassium ($K^{42}$ space) when compared with matched controls. Three of the 5 subjects with hyperaldosteronism and one of 5 with Cushing's Disease showed elevations in exchangeable body sodium ($Na^{24}$ space) and extracellular fluid volume ($S^{35}O_4$ space). Dietary restoration of body potassium to normal levels was not associated with reductions in blood pressure.

Following adrenal surgery, reduction in blood pressure and rise in body potassium were observed in all. Two subjects with hyperaldosteronism and one with Cushing's Disease had a sodium diuresis and comparable decreases in body sodium and ECF volume; in others, however, postoperative reductions in blood pressure occurred without significant changes in these measurements. Administration of the steroidal antagonist SC-8109 (Searle) [19-nor analog of 3-(3-oxo-17$\beta$-hydroxy-4-androsten-17$\alpha$-yl) propionic acid $\gamma$-lactone] to a patient with Conn's syndrome resulted in marked sodium diuresis and slight potassium retention without a fall in blood pressure.

Chronic administration of desoxycorticosterone acetate or 9-$\alpha$-fluorohydrocortisone to 8 normal and 2 splanchnicectomized normotensive subjects produced significant rises in exchangeable sodium and ECF volume and reductions in exchangeable potassium. Six of the subjects developed significant elevations in blood pressure; addition of SC-8109 to one of these subjects produced a sodium diuresis and parallel reduction of exchangeable sodium towards pretreatment levels, but with maintained hypertension.

In conclusion: (1) "steroid hypertension" is associated with reduction in body potassium and occasional elevations of body sodium and ECF volume; (2) elevation of blood pressure may persist despite correction of body sodium and potassium by dietary measures or steroid

antagonists; (3) reduction in blood pressure following adrenal surgery may occur without changes in body sodium and ECF volume.

### Further Studies on Urinary Aldosterone in Human Arterial Hypertension

By *Jacques Genest, Erich Koiw, Wojciech Nowaczynski and Gilles Leboeuf.* Clinical Research Department, Hotel-Dieu Hospital, Montreal. (Aided by grants from the Life Insurance Medical Research Fund, the Federal-Provincial Public Health Service and the Ciba Co., Montreal.)

The work described below represents a continued research in the participation of the adrenocortical hormones in human arterial hypertension. Thirty-three urinary aldosterone determinations were performed in normal subjects with the use of a new chemical method previously described. The mean excretion was 3.6 $\mu$g. $\pm$ 0.42 per day with a range of 1 to 9.5 $\mu$g. per day. Sixty-one urinary aldosterone determinations were performed in 40 patients with essential, renal and malignant hypertension, without cardiac failure and maintained on unrestricted diets. The mean excretion in each of these three groups was twice the normal mean ("p" $< 0.001$). Fifty-five % of these patients had urinary aldosterone excretion higher than normal or in the higher limit of the normal range. Eleven serial urinary aldosterone determinations in a normal subject on usual activities and self-selected diet showed values below 6.5 $\mu$g./day despite variations in urinary sodium from 66 to 386 mEq./day. On the other hand, 3 patients with early hypertension and on unrestricted diets showed marked fluctuations in urinary aldosterone excretion. Patient A with asymptomatic essential hypertension had an aldosterone excretion of 7, 15.7, 3.6, 9.3 and 14 $\mu$g./day. Patient B with benign essential hypertension and minimal symptoms had urinary aldosterone values of 10.2, 1.2, 1.6, 15.5, 14.5, 4 and 8.8 $\mu$g./day. Patient C with asymptomatic renal hypertension showed similar variations of 12.1 (average of 2 days), 2.4, 4.5 and 7.2 $\mu$g./day. No reasons of acute stress, anxiety state, marked changes in sodium or potassium intake, presence or absence of exertional dyspnea could be found to account for such variations in these 3 patients. These findings bring additional direct evidence for an adrenocortical disturbance in human essential hypertension.

### Susceptibility of Rats with Hormonal Hypertension to Experimental Pyelonephritis

By *James W. Woods.* Department of Medicine, University of North Carolina School of Medicine, Chapel Hill.

Ureteral ligation, mechanical trauma, scar formation after staphylococcal infection, and localized thermal injury to the renal medulla have been shown to predispose to the development of coliform pyelonephritis in the experimental animal. Other renal insults such as nephrosclerosis and necrotizing arteriolitis may act similarly. In humans, pyelonephritis may be superimposed on hypertensive disease more frequently than is at present suspected. The present investigation was designed to test this hypothesis.

Female Sprague-Dawley rats weighing 45–60 Gm. had left nephrectomy, 3–4 fortnightly injections of desoxycorticosterone trimethylacetate (DCA), and drank 1% NaCl. Potassium depletion was prevented by supplementary KCl as shown by muscle analyses. Control animals had left nephrectomy, but drank tap water and received no DCA. Experimentals and controls were pair-fed on Ralston Laboratory Chow. Weight curves were practically identical. Systolic blood pressure was indirectly determined in the unanesthetized state by means of a microphonic manometer. The DCA treated animals became hypertensive within 2–3 weeks and developed diffuse arteriolarsclerosis, most evident in the kidney, soon thereafter.

The animals were then inoculated intravenously with 1 ml. of a suspension of E. coli containing 75–150 million organisms. Twenty-three of 33 hypertensives, but only 3 of 33 controls, developed varying degrees of pyelonephritis.

These data support the interpretation that DCA- and saline-induced hypertension renders rats more susceptible to hematogenous renal infection.

### Studies on Enzymatic Action of a Renin Preparation

By *H. G. Langford.* Department of Medicine, University of Mississippi Medical Center, Jackson. (Aided by a grant from the National Heart Institute, USPHS.)

Skeggs has presented evidence to show that renin acts on renin-substrate to split a leucyl-leucine linkage, thus releasing angiotensin. The present study was designed to study this enzymatic action on di- and tripeptides, then with the information obtained return to the natural substrate and ask the appropriate questions.

Hog renin was incubated 2 hours at pH

7.4, 37 C. with the appropriate substrate. After ETOH precipitation, samples of the filtrate were chromatographed overnight in an ascending butanol-ethanol-water system, and the spots developed with ninhydrin.

In confirmation of Skeggs findings, leucyl-leucine was split completely. To determine if this splitting was due to renin, its properties were compared with those of L A (leucine aminopeotidase), a dipeptidase found in the kidney. The following similarities were found: (1) ability to split L-alanyl-1-leucine, L-leucyl-glycine, and failure to split 1-glycyl-leucine; (2) inhibition by 0.1 M Versene and 0.01 M cyanide. The following differences were found: (1) the pH range is much wider for the renin preparation; (2) and it, unlike L A, will not split L-leucyl-glycyl-glycine.

It was tentatively concluded that the splitting of leucyl-leucine by this preparation was an action of renin itself. If this is so, then the action of renin upon renin substrate should be blocked also by adequate Versene. Preliminary experiments, with assay of the angiotensin formed in the anaesthetized dog, suggest this is so. This suggests that renin is a metallo-protein.

### The Question of Vascular Hyper-responsiveness in Arterial Hypertension

By *Paul D. Redleaf and Louis Tobian.* Department of Medicine, University of Minnesota, Minneapolis.

Hyper-responsiveness of arteriolar smooth muscle to normal amounts of pressor substances has been invoked to account for the increased arteriolar resistance which characterizes hypertension. Supporting this hypothesis, less norepinephrine is required to produce a given decrease in blood flow in the nailfold, the conjunctiva, or the hand in hypertensive than in normotensive patients. However, in arterioles with an increase in wall thickness due to waterlogging or hypertrophy, a given degree of muscle shortening will cause an exaggerated increase in resistance to flow. Such an increase in wall thickness probably occurs in human hypertension. Moreover, it can be calculated by using models that a decrease in lumen size per se as a result of already shortened circular muscle will cause an exaggerated increase in flow resistance when additional muscle shortening occurs.

The use of helical strips of aorta eliminates the "apparent hyper-responsiveness" which could occur as a result of waterlogging, muscular hypertrophy, or pre-existent contraction. Such spirals were prepared from 26 normotensive and 29 hypertensive rats. The latter included 6 subgroups in which the hypertension had been produced by different methods or had existed for various durations. After incubation of the spiral at 0.5 Gm. tension for 3 hours, norepinephrine was added to the muscle bath and the resulting increased tension was measured isometrically. The aortic strips from 26 normotensive rats developed a mean additional tension of 0.45 Gm. with norepinephrine. Strips from 19 hypertensive rats developed identical increments of tension. Strips from 10 other hypertensive rats developed less tension than did any strips from normotensive rats. No hypertensive preparation displayed hyper-responsiveness to norepinephrine.

Muscle of the aorta of rats may or may not be typical of all arterial smooth muscle. If it is similar, our results suggest that the "hyper-responsiveness" of arterioles in hypertensive subjects may be "apparent" rather than real.

### The Cardiovascular Response to Norepinephrine in Aortic Regurgitation

By *Timothy J. Regan, Valentino DeFazio and Harper K. Hellems.* Wayne State University College of Medicine, and Detroit Receiving Hospital, Detroit.

Since the factors responsible for control of the regurgitant blood flow in aortic insufficiency have not been clearly defined, a study has been designed to assess the effect of increased sympathetic activity upon the hemodynamics of this clinical state. Six patients with predominant aortic valve incompetence and 5 normals were infused with norepinephrine, 0.1 $\mu$g. to 0.2 $\mu$g./Kg. for 15-minute periods. Brachial artery, pulmonary artery, pulmonary capillary pressures and cardiac output by the dye method were obtained in the control period and after attaining a steady state during norepinephrine infusion. Indicator dilution curves were analyzed for estimation of cardiac output, central volume and valvular regurgitation. The latter parameter was derived by the reciprocal of the downslope method of Korner and Schillingford, which appears to have semiquantitative validity.

The rise in arterial diastolic pressure in aortic insufficiency was slightly less than the response in normals and there was no significant change in pulse rate. The P.A. and P.C. pressures increased, the capillary pressures tripling at the higher infusion level. The calculated central volume increased from 2.65 to 3.40 L., while the aortic output declined slightly (5.5 ± 1.1

to 4.9 ± 1.1 L./min.). A decrease in the ratio of the observed to the predicted slope of the dye curves resulted in an estimated reduction in regurgitant flow after norepinephrine in each case (3.39 L./min. to 1.68 L./min.) (mean diff. = 1.71 ± 1.0). Thus the total cardiac output was reduced.

While the regulation of arteriolar tone and cardiac activity are important responses to norepinephrine, the adjustment in vascular volume may be the most important determinant of the net response. The central displacement of blood from the arterial and venous systems, resulting in a higher pressure-volume relationship in the left ventricle and a reduced arterial volume, offer a tentative explanation for the observed changes.

### The Effect of Chlorothiazide on Norepinephrine Response in Human Hypertension

By *John P. Merrill, Alberto Guinand-Baldo and Carmelo Giordano.* Boston.

Chlorothiazide has been an effective agent in the treatment of human hypertension particularly in conjunction with ganglionic blockade. Its action appears to be enhanced by, but not entirely dependent upon, sodium deprivation. We have investigated the mechanism of Chlorothiazide action in conjunction with ganglionic blockade on high and low sodium diets by a standardized norepinephrine tolerance test and by observation of the response to tilting, the normal response to which has recently been demonstrated to be associated with the release of norepinephrine from the autonomic nervous system. In the control and test situations norepinephrine was infused by a constant infusion pump at 0.04 μg. and 0.2 μg./Kg. body weight/min. Blood pressure, pulse, and in some cases pulse volume curves were measured. The height of the blood pressure response and the time required to reach this response was noted. The data show that the accentuated response to norepinephrine which occurs in the presence of ganglionic blockade was decreased by prior administration of Chlorothiazide. In a typical experiment—before Chlorothiazide mean arterial pressure rise: 55 mm. in 2 minutes; after Chlorothiazide mean arterial pressure rise: 42 mm. in 7 minutes. This decreased response was much more marked on a low sodium diet than on a high sodium diet. The effect of Chlorothiazide in decreasing norepinephrine "ceiling" was apparent when tested without other medication and was enhanced similarly by low sodium intake. Maintenance of diastolic pressure upon tilting for 30 minutes at 60° was impaired following the administration of Chlorothiazide. The possibility that Chlorothiazide may exert its hypotensive action in part by blocking the response of the vasculature to locally elaborated pressor amine stimuli or blocking its release at the effector site is suggested by these results.

### Influence of Local Cation Concentration Variation Upon Small and Large Vessels and Vessel Respones to Norepinephrine and Acetyl-beta-methylcholine

By *Francis J. Haddy, Dean Emanuel and Jerry Scott.* Departments of Medicine and Physiology, Northwestern University; V. A. Research Hospital, Chicago; and U. S. Army Medical Research Laboratory, Fort Knox.

Most systemic hypertensions result from an increase in peripheral vascular resistance. Numerous studies suggest that some hypertensions and hypotensions are related to certain cations. It is known that the responses of nervous tissue and smooth muscle to the adrenalines and to acetylcholine are modified by changes in the concentration ratios of surrounding cations. Some studies indicate that the peripheral vessels of essential hypertensives react to vasoactive substances in an exaggerated manner. For these 4 reasons, the effect of varying cation concentrations upon foreleg vascular resistance has been studied in 55 pentobarbitalized dogs. Foreleg blood flow rate was kept constant with a pump interposed in the brachial artery. Pressures were measured in the brachial artery, a foot pad small artery, a paw small vein and the cephalic vein. From these values, total, artery, small vessel (mainly arteriolar) and venous resistances were calculated. Various $Na^+$, $K^+$, $Mg^{++}$ and $Ca^{++}$ salts were infused into the brachial artery at rates sufficient to raise concentrations only in the leg. Norepinephrine and acetyl-beta-methylcholine were injected into the brachial artery before, during and after infusions. $[H^+]$ and $CO_2$ tension were varied by respiratory mechanisms and the local effects studied with the leg denervated and phentolaminized. Progressive elevation of serum $[K^+]$ from 3 to 8 mEq./L. resulted in a progressive total resistance decrease due to small vessel dilatation. Further elevation invoked superimposition of artery constriction upon small vessel dilatation. Total resistance decreased as a function of $[Na^+]$, $[Mg^+]$ and $[H^+]$ over ranges compatible with life. The resistance de-

creases were due, primarily, to small vessel dilatation. Elevation of serum [Ca++] increased total resistance by small vessel constriction. Total constrictor and dilator responses to norepinephrine and acetyl-beta-methylcholine, respectively, were depressed during sodium and potassium infusions. These studies, regardless of their implications to the tensionopathies, show that cations have an important local effect upon peripheral vascular resistance and upon vessel responses to vasoactive substances.

### Hemodynamic Effects of Tetraethyl Ammonium Chloride in Hypertensive and Normotensive Subjects during Levarterenol Infusions

By *Harold A. Shafter, Harry A. Bliss and Adrian M. Ostfeld.* Departments of Medicine and Preventive Medicine, University of Illinois College of Medicine, Chicago.

This study investigated mechanisms for buffering hemodynamic effects of levarterenol in normotensive and hypertensive subjects.

Subjects were in the postabsorptive state. BP and heart rate (HR) were recorded by arterial puncture and strain gauge manometer. Cardiac output (CO), mean circulation time (MCT) and "central blood volume" (CBV) were determined from an indicator dilution curve using $I^{131}$-tagged albumin. Right atrial (RA) pressure was recorded in 6 subjects by cardiac catheter and strain gauge manometer. After control measurements, levarterenol 10 μg./minute was infused intravenously. After 10 minutes, measurements were repeated. Tetraethyl ammonium chloride (TEAC) 6 mg./Kg. was then infused intravenously in 1 minute. Levarterenol infusion was continued and measurements were repeated after 4 minutes.

Two hemodynamic patterns were discernible after TEAC. In 2 hypertensives and 3 normotensives, CO, mean BP, HR and CBV increased significantly and total peripheral resistance (TPR) decreased significantly. In 3 hypertensives and 2 normotensives there was no significant change in CO, mean BP, CBV or TPR. In 9 of 10 cases MCT and in 5 of 6 cases RA mean (or "Z" point) pressure decreased. For the entire group, there was significant positive correlation ($p < 0.01$) of CO with HR, mean BP, and CBV, and significant negative correlation of CO with TPR and MCT. "Partial $r$" values indicated that correlation between HR and CO was independent of variations in TPR or CBV.

Factors increasing HR were closely related to factors increasing CO, whereas increased right ventricular end-diastolic tension was not implicated. During levarterenol infusion, TEAC blockade of autonomic control of heart rate and contractility and of peripheral vasometer control allowed a rise in CO in some individuals. In others, a presumably equal degree of blockade was unaccompanied by significant hemodynamic changes. Autonomic buffering may differ quantitatively or qualitatively in the 2 groups.

### Methindethyrium-Cryptenamine-Reserpine Therapy of Ambulatory Patients with Arterial Hypertension

By *Burton M. Cohen.* St. Elizabeth's Hospital, Elizabeth, New Jersey.

Methindethyrium chloride is an asymmetrical bisquaternary ammonium salt closely related to the ganglionic blocking agents. This report summarizes the clinical response of 21 patients with arterial hypertension of Smithwick Grades III and IV to oral therapy with methindethyrium, cryptenamine and reserpine in a fixed ratio.

Tablets containing 100 mg. of methindethyrium, 1 mg. of cryptenamine and 0.08 mg. of reserpine were administered to 12 women and 9 men on an out-patient basis for periods averaging 15 weeks of continuous observation.

The final average oral daily dose requirement was 3.5 tablets for the group as a whole. There was a decrement of average mean arterial blood pressure in the supine position of 35 mm. of mercury; 6 patients achieved a treatment diastolic pressure of 90 mm. of mercury or less. Orthostasis was not significant. The addition of chlorthiazide to the program resulted in a further lowering of blood pressure "floor."

Sixteen of the 21 patients reported no untoward effects that could be related to therapy. Urinary frequency, oral drying and visual blurring were infrequent. In no instance was it necessary to discontinue therapy because of limiting side-reactions. "Hypertensive headache," precordial discomfort and palpitation, and dyspnea were lessened. Early objective improvement was limited to regression of proteinuria and decrease in nitrogen retention; effects on heart size, funduscopic abnormalities and the electrocardiogram were not seen.

These preliminary data suggest the usefulness of methindethyrium, cryptenamine and reserpine when employed in concert in a fixed tablet combination in the office or out-clinic therapy of

patients with moderately severe arterial hypertension.

### Preliminary Experience with a New Ganglionic Blocking Agent: (Wyeth, WY-1395)

By *D. Edmond Miller, Morton D. Bogdonoff and Dawn F. Reed.* Department of Medicine, Duke Medical Center, Durham, North Carolina. (Aided by a grant from the Wyeth Laboratories.)

Search for the ideal ganglionic blocking drug, possessing the characteristics of consistent absorption, prolonged action, and infrequent tolerance and side effects, prompted a clinical evaluation of Camphidonium (Wyeth, Wy-1395), an asymetric bis-quaternary amine, in the therapy of arterial hypertension.

Nine ambulatory patients with moderate to severe long-standing hypertension (mean systolic pressure 209 mm. Hg (S.D. ± 37); mean diastolic pressure 124 mm. Hg (S.D. ± 19) were treated with WY-1395 alone for 2 to 4 months. Four had Grade I retinopathy; three, Grade II; two, Grade III. Two-hour PSP excretion averaged 64% (23–90%). The hypertension was essential in 5, secondary to pyelonephritis and/or preeclampsia in 4. Five had responded poorly to previous regimens, including other ganglionic blocking drugs in 3. In the remainder, therapy was inadequate or had been discontinued.

Forty to 80 mg. in 2 divided doses, p.o., a.c., in 9 erect patients, were accompanied by mean reduction of systolic pressure of 60 mm. Hg ($p < .001$), diastolic pressure of 22 mm. Hg ($p < .01$). Supine pressures were unpredictably lowered. None failed to respond to the drug.

Maintenance dosage had to be gradually increased in 6, indicating tolerance; unpredictable lowering of pressure occurred in 4, indicating erratic absorption. Side effects of dizziness, dry mouth, blurring of vision and photophobia paralleled reduction of pressure. The ocular symptoms were relieved by pilocarpine. Side effects were annoying early in 6, but tolerance for these developed as well. No difficulty with constipation occurred. NPN levels remained normal. No progression of the hypertensive vascular disease nor toxic manifestations of the drug were observed. Two patients demonstrated reduction of retinal hemorrhages and exudates. Two patients, who briefly discontinued therapy, experienced marked elevation of blood pressure.

Because of the initial favorable experiences with this agent, further studies are being conducted.

### An Evaluation of the Antihypertensive Potency and Side Effects of SU-3118 (Carbthoxysyringoyl Methylreserpate)

By *Fred T. Darvill, Jr.* Northern State Hospital, Sedro Woolley, Washington, and Department of Medicine, University of Washington School of Medicine, Seattle. (Aided by a grant from Ciba Pharmaceutical Company.)

SU-3118 (Ciba), a reserpine derivative, its identical placebo and reserpine were administered by code number to 28 patients with sustained diastolic hypertension (diastolic pressure over 100 mm. Hg). Ten patients (series I) who had never received antihypertensive therapy were evaluated for 12 weeks as follows: first two weeks, no medication; second two weeks, SU-3118 placebo b.i.d.; third two weeks, SU-3118 0.25 mg. b.i.d.; fourth two weeks, SU-3118 0.5 mg. b.i.d.; fifth two weeks, SU-3118 1.0 mg. b.i.d.; and sixth two weeks, reserpine 0.25 mg. b.i.d.

One patient (series II) was given placebo and then 5 mg. and later 10 mg. of SU-3118 intramuscularly. SU-3118 and its placebo were substituted for reserpine in 9 hypertensive patients (series III) receiving reserpine and hydralazine. SU-3118 (0.25 mg. b.i.d.) and its placebo were substituted for reserpine (0.25 mg. b.i.d.) in 8 patients (series IV) receiving reserpine therapy for hypertension. Blood pressure determinations were obtained 3 times a day on all patients. The mean diastolic blood pressure was determined during the last week of each medication period and the results analyzed statistically. Side effects were recorded throughout the study.

In series I, a statistically significant fall in blood pressure was achieved with 1 mg. b.i.d. of SU-3118 orally ($P = .03$) and with oral reserpine ($P = <.005$). In series II, a significant fall in blood pressure ($P = .005$) was achieved with 10 mg. of SU-3118 intramuscularly. In series III no significant differences could be demonstrated between the drugs. In series IV reserpine in equivalent dose was significantly more hypotensive ($P = .04$) than SU-3118. Very mild side effects (nightmares, stomach upset and rhinitis) were reported by only 4 patients.

In summary, SU-3118 exerts a significant hypotensive effect. One mg. of SU-3118 is almost as effective an antihypertensive agent as 0.25 mg.

of reserpine. Side effects are minimal. This drug may be of value in those hypertensive patients unable to tolerate reserpine.

### Blood Radioactivity and Turbidity Patterns after a Radioactive Fat Test Meal in Patients with Coronary Atherosclerosis

By *Asher Woldow, Donald Berkowitz, A. Gerson Jacobs and William Likoff.* Department of Medicine, Albert Einstein Medical Center, Northern Division, Philadelphia.

Serum lactescence after a fat meal has been used by some workers to distinguish normal individuals from abnormals. Abnormal responses characterized by increased postprandial turbidity have been found in a significant percent of patients who have evidence of coronary artery disease. However, in many cases, the test falls short of its expectations.

More recently, radioactive fat tolerance curves done in a group of patients who have recovered from a myocardial infarction have demonstrated certain significant alterations from the normal, i.e., a higher peak blood radioactivity level, and an abnormal retention of activity after 24 hours (Likoff and Berkowitz).

We have compared radioactive fat absorptive patterns with serum turbidity curves in a large group of patients, both normal and with evidence of coronary artery atherosclerosis both with and without elevated cholesterol values.

Significant differences in the results obtained by the 2 technics have been noted. The peak turbidity value usually occurs before the maximum blood radioactivity level is reached, and returns to normal at a time when the blood radioactivity is still increasing.

### Effect of the Hormone Relaxin on the Aorta of the Guinea and Rat

By *J. O'Neal Humphries and Allen L. Pusch.* Department of Medicine, Johns Hopkins University School of Medicine, Baltimore.

Dissecting aneurysm of the aorta in females under 40 years of age is associated with pregnancy in 50% of the reported cases. Hypertension does not appear to be a necessary factor in such dissections. The possibility that relaxin, the "third hormone of pregnancy," is related to this increased incidence of dissection was investigated. Relaxin is reported to produce dissolution and splitting of the collagen, a breakdown of glycoprotein with depolymerization of the ground substance, and an increase in the number of capillaries in the public symphysis. The effects have been studied primarily in the reproductive tract of experimental animals and more recently in sponges superficially implanted in rats. Because relaxin is employed clinically to halt premature labor and to treat schroderma, investigation of its effect on the aorta was considered important.

Twenty guinea pigs were primed with estrogen (100 $\mu$g.) for one week and then given 150 Guinea Pig Units (GPU) of relaxin per day for 6 weeks. The vehicle for relaxin was a 5% beeswax in peanut oil repository. A second group of 20 guinea pigs received the same amount of relaxin without previous estrogen priming while a third group of 20 was primed with estrogen and received no relaxin. Each group contained equal numbers of males and females. The guinea pigs were approximately 3 weeks old at the start of the experiment. Ten animals (5 males and 5 females) from each group were challenged with levarterenol (3 mg. subcutaneously) 60 and 30 minutes before being sacrificed. No gross abnormalities of the aorta or other internal organs were observed except in 2 animals which had pulmonary hemorrhages. Histologic studies of the aortae did not indicate abnormality in any of the 3 groups.

Four rats, primed with estrogen, were treated with relaxin (1500 GPU per day) for 10 days. All died of pulmonary hemorrhage and edema when challenged with levarterenol. There were no histologic abnormalities in the aorta. The aorta from both control and treated animals withstood intraluminary pressure of at least 2000 mm. Hg.

In a third series of experiments, involving 18 guinea pigs, chronic administration of DOCA and levarterenol with estrogen and relaxin was found to have no evident effects on the aorta.

### The Effect of Local Arteriosclerosis upon the Arterial Pressure-Volume Curve of an Extremity of Man Measured In Vivo

By *J. Edwin Wood.* Evans Memorial and Massachusetts Memorial Hospitals, Boston, and Department of Medicine, Boston University School of Medicine. (Aided by grants from the United States Air Force and the U. S. Public Health Service.)

A plethysmographic method for measuring distensibility (pressure-volume curve) of the peripheral arteries has been evaluated as a means

of quantitating the degree of arteriosclerosis present in an extremity.

The change in volume of the calf with each cardiac cycle was measured from an air plethysmograph. Effective intra-arterial pressure was varied from one series of pulse cycles to the next by altering the local counter pressure of the air in the plethysmograph. Changes in volume of the limb with systole at each effective arterial pressure were determined with a sensitive pressure transformer calibrated with a pulse of known volume at each plethysmographic pressure during circulatory arrest. Artefacts due to the presence of poorly pressurized cones of tissue at either end of the plethysmograph were prevented by extending the area of counter pressure along the limb proximally and distally. Changes in limb volume were plotted against changes in effective arterial pressure to give an arterial pressure-volume curve, but for simplicity they are reported here as the total change in arterial volume (per 100 cc. of limb) due to a change of effective arterial pressure of 0 to 80 mm. Hg.

Twenty-nine patients without clinically evident arteriosclerosis of the lower extremity had a mean change in arterial volume of 0.145 cc. ($\sigma = 0.056$) per 100 cc. of limb produced by a change in effective arterial pressure of 0 to 80 mm. Hg. Their ages ranged from 15 to 71 years and 16 were males. Nineteen patients with objective evidence of advanced arteriosclerosis of the lower extremity had a mean change in arterial volume of 0.047 cc. ($\sigma = 0.017$ cc.). Their ages ranged from 35 to 74 years and 17 were males. These results were reproducible on repeated tests in the same subjects.

The method is without discomfort, and appears to be a valid means of following objectively the course of peripheral arteriosclerosis.

### Intraarterial and Intravenous Administration of Agents Used to Induce Peripheral Vasodilation

By *Maria Aurora Antonio, Walter Redisch, Kurt DeCrinis, Aurelius Bogdanovics and J. Murray Steele.* New York University Research Service, Goldwater Memorial Hospital, and Department of Medicine, New York University College of Medicine, New York.

Blood flow responses in the lower extremities to 9 agents were tested in a constant temperature room maintained at 20 C. and 55% humidity in "normals" and patients with obliterative arterial disease. Some limbs had been previously sympathectomized. Intra-arterial administration was compared with intravenous. 50 mg. Priscoline, 7 mg. Arlidin, 25 mg. 27 M.I., 3 mg. Ecolid, 3 mg. histamine, 50 mg. Ilidar, 100 mg. nicotinic acid, 8 mg. diethylaminoethanol, 0.025 mg. Papaverine, and 500 cc. normal saline were administered in repeated single dose experiments. Surface temperature and plethysmographic changes in the leg were recorded. Cardiac output and renal plasma flow were simultaneously measured in 2 intravenous experiments with each drug —one in a normal and one in an arteriosclerotic subject. Arterial pressure and pulse rate are ascertained throughout all experiments.

Priscoline almost uniformly increased surface temperature, while plethysmographic responses were slight and in varying directions. Arlidin produced a slight increase in surface temperature but uniformly marked increase in blood flow to the muscle. Histamine, diethylaminoethanol, Ilidar, Ecolid, Papaverine, and nicotinic acid increased regularly both surface temperature and blood flow to the muscle. 27 M.I. produced no significant change in either measurement. In all subjects responses to the individual agents were uniform in direction regardless of the route of administration. To all agents the "normals" responded more promptly, reaching maximum within 20–30 minutes with total duration of 60–120 minutes in contrast to the arteriosclerotics who had a marked delay in onset of response reaching maximum within 40–90 minutes, while the total duration was the same. Intra-arterial administration produced in both groups an earlier response than the intravenous, but of about equal magnitude and duration. Systemic side effects were less with the intra-arterial than with the intravenous route. Sympathectomy seems to alter the response to those agents which increased blood flow in nonsympathectomized limbs: there was an initial decrease followed by much delayed rise.

### The Local Spread of Intradermally Injected Dye in Edematous and Nonedematous Extremities

By *Sam A. Threefoot.* Research Laboratory, Touro Infirmary, and Department of Medicine, Tulane University, New Orleans. (Aided by grants from National Institutes of Health, Louisiana Heart Association, J. A. Hartford Foundation and J. Aron Foundation.)

As part of experiments designed to study the role of lymphatics in edema formation by visualization and cannulation of the lymphatics, serial photographs of the spread of 0.1 ml. 11% patent

blue V injected intradermally were taken at intervals of a few seconds initially and of 10 to 15 minutes later. Areas of dye spread were determined by planimetry of projected enlargements of the photographs.

In addition to differences in configurations of wheals and absence of distinct streamers in edematous extremities described previously by others, time courses of dye spread are also significant. The areas increased most during the first minute of all studies and were greatest in edematous extremities. At 1 minute they averaged 1 cm.$^2$ (0.4 to 1.75) in control extremities and 9 cm.$^2$ (1.06 to 35.5) in edematous extremities; at 10 minutes 1.75 and 14 cm.$^2$; at 100 minutes 4.5 and 20 cm.$^2$ Some subjects restudied both with and without edema or in both edematous and nonedematous extremities demonstrated similar differences, although there was only rough correlation between degree of edema and magnitude of spread.

Although the rapidity of spread in edematous extremities suggested direct interstitial diffusion, much of the increased area was observed to be in discrete, distinct channels, prescribing sharp boundaries for any such interstitial spaces. Dye in lymph and blood obtained by cannulation of discrete channels identified both lymphatics and capillaries as routes of spread. Decreased rate of urinary excretion of dye (not attributable to diminished renal function) further suggested that increased spread in edematous extremities was into spaces not directly communicating with the circulating system or that engorgement of local vessels produced pools into which diffusion occurred.

These data indicate increased dispersion in, but decreased flow from, an edematous extremity.

### Pathogenesis of Morphologic Lesions Due to Serotonin

By *Richard A. MacDonald, Stanley L. Robbins and G. Kenneth Mallory.* Mallory Institute of Pathology, Boston City Hospital; Departments of Pathology of Harvard Medical School, Boston University School of Medicine; and Tufts University School of Medicine. (Aided by grants from the American Heart Association and the Boston Chapter of the Massachusetts Heart Association.)

In humans with metastatic carcinoid tumor, serotonin has been associated with cardiac valvular fibrosis and peptic ulcer. In the present experiment, studies were made in rats of the occurrence and pathogenesis of morphologic lesions due to acute and chronic administration of serotonin.

In acute studies, 96 rats were used; autopsies were performed at 2-hour intervals up to 24 hours following single subcutaneous or intraperitoneal injections of 0.1, 8 and 72 mg. of serotonin creatinine sulphate and 5-hydroxtryptophane. Vascular studies were made using India ink injections at time of sacrifice. In chronic studies, 142 rats were used; autopsies were performed at monthly intervals. Eight and 16 mg. quantities of serotonin creatinine sulphate were administered subcutaneously twice daily for 15, 30, and 353 consecutive days.

In acute studies, lesions occurred only with 8 and 72 mg. doses, consisting of renal tubular necrosis and gastric mucosal erosions. India ink injections demonstrated vascular spasm in 2 areas: (1) regions corresponding with intralobular arteries and afferent arterioles, although glomeruli filled well, and (2) postglomerular arterioles and capillaries. Postglomerular ischemia preceded morphologic lesions and corresponded in location with areas of proximal tubular necrosis found in animals sacrificed at later time intervals. Gastric mucosal erosions occurred inconstantly and only in the glandular, highly vascular portions of stomach. Similar lesions were occasionally found in control animals given epinephrine. In chronic studies, dermal fibrosis occurred at injection sites, with similar but less marked fibrosis in control animals receiving epinephrine. Renal tubular lesions occurred, which were related to the tubular necrosis found in acute studies. Cardiac valvular changes were not observed.

It is concluded that in rats, morphologic lesions due to serotonin occur only with large, unphysiologic doses, and these changes appear to be secondary to vasoconstrictive and ischemic effects of serotonin.

### Serotonin and Norepinephrine in Bananas

By *T. Phillip Waalkes, Albert Sjoerdsma, Cyrus R. Creveling and Sidney Udenfriend.* National Heart Institute, Bethesda.

Following a report that ingestion of bananas produces an increased urinary excretion of the serotonin (5-hydroxytryptamine) metabolite, 5-hydroxyindoleacetic acid, chemical studies were undertaken on the banana to explain this phenomenon. Extracts of bananas were prepared

and examined by chromatographic, spectrophotofluorometric and bio-assay methods. It was found that the edible portion of a single banana contains approximately 4 mg. of serotonin, comparable amounts being found in the skin. Further work led to the surprising finding that the banana also contains *L*-norepinephrine (ca. 0.3 mg. in the edible portion) and other catecholamines. Although 5-hydroxyindolamines have been found in plants previously, they have usually been associated with plants regarded as toxic to animals. To our knowledge this is the first report of the finding of norepinephrine in plant material.

Whether the oral administration of these amines through banana feeding can have effects on the gastrointestinal tract or cardiovascular system remains to be determined. One might speculate as to whether some of the reported therapeutic uses of bananas (in celiac disease, peptic ulcer, constipation, etc.) may be due to the presence of these amines. As a result of these findings with bananas, we were encouraged to administer large amounts (15–20 mg.) of the amines to patients orally. No untoward effects were observed.

Of immediate clinical significance is the fact that ingestion of bananas may lead to erroneous chemical diagnoses of carcinoid tumors and pheochromocytoma by producing increased urinary excretion of serotonin and norepinephrine metabolites. Also, bananas should be eliminated from diets of patients whose urinary indoles and catecholamines are being measured for other purposes, e.g., mental disease. It remains to be determined whether other edible plants contain these agents.

## CONNECTIVE TISSUE

### Biochemical Aspects of the Papain-Induced Reversible Changes in Rabbit Cartilage

By *Theodore T. Tsaltas.* Department of Pathology, New York University College of Medicine, New York.

The intravenous injection of papain in rabbits produces reversible collapse of the ears, and microscopically, profound depletion of the basophilic cartilage matrix (L. Thomas). The biochemical aspects of these changes have been investigated by microchemical and tracer ($S^{35}$) studies.

Thus, 24 hours after the injection of papain, the amount of mucochondroitin sulfate (M.C.S.) isolated from the ear cartilage was 35–40% lower than that in the control animals. The $S^{35}$ activity in the isolated M.C.S. decreased by approximately 30%. Concommitantly, the $S^{35}$ activity in the organic and inorganic fractions of the serum increased considerably, the most marked being the increase in the organic fraction. The urinary excretion of glucurenic acid and $S^{35}$-containing compounds increased in the same period many fold.

In a large series of experiments, animals were sacrificed at regular time intervals from 2 to 72 hours after the injection of papain, in order to follow the rate of these changes. The maximum depletion of M.C.S. from cartilage occurred 12 hours after the injection. This depletion was maintained for 24 hours, thereafter increasing gradually to normal levels. The $S^{35}$ activity in the serum organic and inorganic fractions reached a maximum at the same time when the depletion of cartilage M.C.S. was maximal.

A very important change was observed in the $N_2$ : Hexozamine ratio of the M.C.S. This increased markedly and reached the highest point 24 hours after the injection of papain and returned to normal levels 60 hours later.

These experiments suggest that the microscopic changes are due to the removal of M.C.S. from the cartilage, with resulting increase of $S^{35}$-containing compounds in the serum. The eventual replenishment of the cartilage with M.C.S. offers a possible explanation to the restoration of the cartilage to its original gross and microscopic properties.

### Pseudoxanthoma Elasticum: Clinical Findings and Identification of the Anatomic Defect

By *Gerald P. Rodnan, Edwin R. Fisher and Joseph E. Warren.* Departments of Medicine and Pathology, University of Pittsburgh, Pittsburgh.

Pseudoxanthoma elasticum (PXE) is a heritable disorder of connective tissue which commonly affects the skin, fundus oculi and vascular system. While the bandlike agglomeration of hematoxylinophilic curlicues and granules

in the midcorium which comprises the dermal lesion is well recognized, there has been considerable controversy concerning the origin of the affected tissue. It has been suggested recently (McKusick, 1956) that the primary disorder is one of collagen, rather than of elastic tissue as was originally believed. To examine this matter, skin biopsies were obtained from a sibship of 3 patients (sisters, ages 49 and 35, brother, 47) and from 4 unaffected members representing 3 generations of the family. Each of the patients exhibited typical skin changes (wrinkling, softening, inelasticity) in flexural folds. Each had angioid streaking of the retina and in the 2 older individuals retinal hemorrhages had produced visual impairment. There was in all 3 marked weakness of the peripheral arterial pulsations.

Sections of skin were subjected to histochemical study and fluorescence and electron microscopy. These revealed marked similarities between the fibrillary and granular component of the lesion and normal elastic fibers. Particularly striking in this regard were their brilliant autofluorescence, lack of periodicity in ultramicroscopic preparations, lability to elastase, and inhibition of their affinity for elastic tissue dyes following methylation. No lesions were found in the biopsies taken from the patients' parents and other members of the kindred who exhibited no clinical evidence of disease. In one of the patients, however, a typical lesion was noted in a normal-appearing area of skin far distant from any obviously involved site.

It is concluded that the primary defect in PXE consists of an abnormality of elastic tissue, rather than of collagen, and that flexural stress is of importance in the clinical localization of the cutaneous lesions.

**A Study of the Fine Structure of the Spleen in Experimental Amyloidosis of the Rabbit**

By *Alan S. Cohen, Leon Weiss and Evan Calkins.* Medical Service, Massachusetts General Hospital, Departments of Medicine and Anatomy, Harvard Medical School.

The spleens of rabbits with experimentally induced amyloidosis were examined with the electron microscope to determine the earliest site of deposition of the amyloid, the manner of its development and its structual organization. The disease was induced in 12 rabbits by subcutaneous injections of casein, as previously reported. Animals were sacrificed at 0, 2, 4, 6 and 8 months, and portions of the spleens were fixed in osmium tetroxide and embedded in methacrylate. Thin sections were cut on a Porter-Blum microtome, and examined in an RCA-EMU-2E microscope. Successive thick sections were cut and stained for light microscopy.

The amyloid, so induced, bound Congo red, exhibited metachromasia with methyl violet and was eosinophilic in the light microscope. It appeared initially in the reticulum (i.e. connective tissue ensheathing the endothelium of the splenic sinuses) of the marginal zone of the red pulp. The process culminated in massive replacement of the red pulp and finally white pulp by the seemingly amorphous material.

In the electron microscope, thickening of the subendothelial reticulum was the first change observed. Amyloid then appeared to accumulate progressively until it formed large islands separated by cytoplasmic strands. Endothelial cells were stretched over the masses of amyloid, separating them from the sinusal lumen. Spleens which appeared to be almost totally replaced by amyloid by light microscopy were surprisingly cellular at high magnification. The organization of continguous cells was well maintained.

The amyloid had a granular appearance not unlike normal reticulum. At high magnification it was complex, containing granular and fine filamentous components and many cytoplasmic projections.

Thus, casein-induced amyloid in the rabbit spleen was first deposited in the connective tissue surrounding the walls of venous sinuses. In the early stages, the amyloid was separated from the sinusal lumen by the cytoplasm of the endothelial cells. The high resolution studies revealed that the amyloid substance had a more complex organization than heretofore suspected.

# ECOLOGY

## Prevalence of Various Illnesses in a "Normal" Population Sample

By *Thomas W. Mou and Harry A. Feldman.* Department of Preventive Medicine, State University of New York, Upstate Medical Center, Syracuse. (Aided by a grant from the National Institutes of Health.)

In a study of the prevalence of various illnesses, 795 families (2,962 persons), randomly selected from certain Syracuse census tracts, were interviewed concerning their previous experiences with a number of medical and surgical diseases. All of this "normal" population was ambulant and 803 individuals were bled for concurrent serologic studies. The historical data, which has been analyzed according to illness, age of occurrence and comparative sex frequency, will be reported at this time.

Appendectomies had been experienced by 346 persons; they were more frequent in females. Incidental appendectomies occurred in the later years much oftener in women.

Fractures were reported by 22% of the males and 16% of the females. In both sexes, 75% of the fractures represented a single experience. The predominance of fractures in young males was negated somewhat by increased mishaps in elderly women.

Poliomyelitis was reported by 36 persons, all in the first 3 decades of life. Varicella, rubeola, rubella and mumps, as expected, occurred predominately in childhood, but approximately 25% of the sample could not recall having had any of these illnesses.

Jaundice had been noted by 105 persons and with equivalent frequency by both sexes. The number of cases declined steadily after the 3rd decade.

Sixty-seven persons stated that they had had rheumatic fever and an additional 81 thought that they might have had it. The rate for "definite" rheumatic fever was 3 to 4 times as high under age 20 as over. Both sexes seemed to be affected equally.

Allergic reactions to penicillin were reported by 52 persons, few of whom were under age 20. The incidence was similar in both sexes until about age 50, when the rate appeared to increase in females.

The diseases selected for this summary are illustrative of those investigated. All have been analyzed so as to yield valid data for comparison with one another. The results are not at variance with accepted impressions, illustrate an approach to the medical definition of a community and will serve as a baseline for future studies of illness trends in the same community.

## Studies in Human Ecology: Perceptions of Life Experiences as a Determinant of the Occurrence of Illness

By *William N. Christenson, Francis D. Kane, Harold G. Wolff and Lawrence E. Hinkle, Jr.* Study Program in Human Health and the Ecology of Man, Departments of Medicine and Psychiatry, New York Hospital-Cornell Medical Center, New York.

Man's adaptation to his environment may involve a wide variety of physiologic processes, known to affect the course of certain diseases.

To assess the effect of such adaptations upon illness in general, Hungarian refugees, a group required to make many major adaptations during the past two decades, were studied; a sample of 75 were selected without regard to health. The occurrence of illness was assessed by a standardized, chronological tabulation of illness episodes, using data derived from a comprehensive, questionnaire-guided medical history and examination. The subject's perceptions of his environment were derived, independently of illness data, from a detailed life history, family history and description of cultural background, social milieu and personality characteristics, assembled during 2 days of observations, interviews and tests, by an anthropologist, sociologist, psychiatrist, psychologist and internist. Using transcriptions of these data, 3 evaluators independently estimated each subject's perceptions of his environment, rating the over-all perception for each year after age 12 on a 5-point scale, ranging from "highly satisfactory" to "highly unsatisfactory."

Estimates of the evaluators correlated well with each other ($r = +0.6$ to $0.7$, $P < 0.01$, $n = 248$ years).

For all subjects, illness episode rates were significantly higher during years when the environment was perceived as unsatisfactory ($P < 0.01$). This higher incidence involved nearly all of the varieties of illness occurring in these subjects, regardless of type, severity or etiologic

category. Only a minority of the new episodes could be attributed primarily to changes in directly acting physical features of the environment; the majority appear to have been initiated, or facilitated, by physiologic changes occurring as a part of the subject's reactions to life situations perceived as frustrating, threatening, overdemanding, or conflict-laden.

The over-all illness episode rate of a subject was an expression of his interaction with his more immediate environment as well as of his inborn and early-acquired characteristics; moreover, the reaction to his perception of his social and interpersonal environment was a most important determinant of variations in the occurrence of illnesses of all types.

# EDUCATION

### A Teaching Method for Problems Involving Doctor-Patient Interaction

By *Albert Stunkard*. Departments of Psychiatry and Medicine, University of Pennsylvania School of Medicine.

This report describes a group-teaching method for problems involving the doctor-patient interaction. Several days before the exercise, short case reports are distributed which describe the patient and the therapeutic situation. At the beginning of the exercise the class discusses the situation and attempts to define the problem(s) presented by the patient, as well as possible solutions. One student who has studied additional information about the case then plays the role of the patient in a series of interviews by his colleagues. Each interview proceeds until difficulty is encountered, at which point the interaction of doctor and patient is discussed, both participants are questioned and new hypotheses are formulated. Another student then resumes the interviewing, and this sequence is repeated until a definition of the problem(s) is reached and a plan of management evolved.

This method provides a laboratory setting for the study of a number of processes involved in the interaction of doctor and patient. By furnishing a standard therapeutic situation it allows the students to observe the effects of a variety of approaches, and to assess the strengths and weaknesses of each. The role-playing situation demonstrates how each physician's interaction with the patient is determined by his own unique characteristics, and how his effectiveness derives from his skillful use of these characteristics.

The method appears widely applicable. Cases can be prepared to illustrate many problems of medical practise, and matters which have been considered teachable, if at all, only in a preceptor relationship, can be profitably approached in a group setting.

### Panel Methods in the Medical Curriculum

By *John C. Rose and Bruce I. Shnider*. Georgetown University School of Medicine, Washington, D. C.

Teaching methods in medical schools are currently the subjects of widespread re-evaluation. This study concerns an analysis of one technic—the panel conference. The merits of simultaneous teaching by several disciplines, as opposed to the lecture method, are not argued. Rather, the mechanics of panels are stressed.

A faculty critique committee evaluated a series of extremely varied 2-hour panels (3 to 6 participants) for third-year students. Sources of data included faculty auditors' reports, student comments and faculty discussion. (Tape recordings were available for analysis.) Areas of consideration outlined to the general faculty follow.

*Responsibility.* The appointed moderator must determine the specific scope and technic of the panel. (Is the subject to be "covered," or are general principles to be stressed? Is controversy to be encouraged?) He must arrange a pre-panel meeting (sometimes two) for organization and prevention of overlap.

*Technic.* The spectrum ranges from a series of lectures to a 2-hour question-answer period. Consciousness of timing and anticipation of the possible extent of student participation are essential. Moderator's participation may be recessive, but his control of the panel must be protected from domineering teachers. Panel size is an important factor. More than 4 panelists may detract from total effectiveness.

*Personal conduct.* Students and faculty auditors specifically scored "harping" about lack of time, exhibition of boredom during other panelists' presentations, lack of awareness of acoustical characteristics of conference rooms, mishandling of pointer light and other small but significant reminders for teachers.

Over 120 faculty members (representing each department in the school) have participated in this "training program," in anticipation of the extensive use of panels in a revised curriculum. Faculty training is essential for the success of this technic. Faculty personal interrelations are improved, duplication in the curriculum avoided, and correlation of basic and clinical sciences advanced.

### Evaluation of a Medical Student Research Program

By *William P. Nelson,* III. Albany Medical College.

At the Albany Medical College there has been a remarkable interest and activity in medical student clinical and basic science research in the last several years. The number of potential candidates for research has doubled in a 2-year period and has surpassed the available financial support. Strenuous efforts to increase fiscal resources have only been partially successful, while certain apparently obvious sources have been unresponsive.

Advantages have probably accrued as a result of the increased numbers of applications, resulting particularly in more specific criteria for the selection of fellows; both past scholarship and anticipated potential for future successful research activity is more critically evaluated. Since students should not work merely as assistants or technicians, the proper selection of the research problem and of the instructor-collaborator is of prime importance and raises important questions regarding how medical students should be introduced to the potential of experiencing a research activity. The time-scheduling of student research introduces problems regarding whether such pursuits can profitably be performed concurrently with other academic assignments, whether they should be integrated separately within the winter academic calendar, or whether such activity should be relegated to summer vacation months. The types of outlet for public recognition of student investigation require attention because intramural publications, student research meetings and acknowledgement of effort by local professional groups, in addition to standard publication of really outstanding work, are important catalysts to student interest and achievement.

Finally, one must weigh the potential advantages and gains in student research against the cost of such programs. Success must be measured not only in terms of tangible results, but by the impact upon the student of the academic philosophy engendered by such activity.

### Research as Part of the Medical Curriculum

By *Vernon W. Lippard and Arthur Ebbert, Jr.* Yale University School of Medicine, New Haven.

Student research is an integral part of the curriculum at Yale University School of Medicine. This report reviews the experience with student research programs at Yale over the past 118 years and describes the program as it is currently organized.

The presentation of a dissertation based upon original investigation is a requirement at this school for graduation. The student is expected to conceive his own research problem and to select the faculty member with whom he will work. This may be done in the first, second, or third year. Once the field of investigation has been decided upon, there follows an exploration of the pertinent literature and conferences with the faculty supervisor until a protocol is developed. At this point, the student is assigned space in his supervisor's laboratory, and a close working relationship is maintained throughout the course of the investigation. The curriculum has been arranged to provide free time for student research in each year. Vacation periods may also be used.

Analysis of student investigations over the past 5 years reveals a fairly even distribution between basic medical science and clinical fields and also that the large majority involved laboratory research. Twenty-nine per cent of the investigations during this period have been published in scientific journals.

It is our contention that opportunity to explore critically the literature in a limited field, to learn something of the experimental method, to bring an investigation to conclusion and write a report, to know the strengths and limitations of biological research, to have curiosity stimulated, and to work intimately with the faculty enhances the competence of a physician. We do not believe that the same results can be obtained when research opportunities are presented on an elective basis.

### Stimulation of Student Interest in Research by a Tutorial Program

By *Claude A. Villee.* Harvard Medical School, Boston.

A tutorial program for undergraduate medical students was established in 1923 at Harvard Medical School. This has been continued to the present except during the acceleration of the curriculum in the war years. Over the years, the tutorial program has undergone a number of changes, but the aim of stimulating interest in research has remained unchanged. Since 1947, tutors in medicine, surgery and the preclinical sciences have held small informal seminars with students of the three upper classes at weekly or biweekly intervals. These afford an opportunity for a student to present to his peers the results of his experiments so far, and what he proposes to do in the future. Other members of the group offer suggestions for revisions of the procedures, additional controls or perhaps some different experimental approach which may be advantageous. Some groups have held miniature symposia on some topic or current interest in which the topic is reviewed from the standpoint of the basic sciences which bear on it and from the viewpoint of the clinical applications of the topic.

Students have been able to carry on research in the small amount of spare time available during the second and third years and in the summers. Since its inception in 1923 the tutorial program has been able to grant to any member of the fourth year class in good academic standing the privilege of substituting up to 6 months completely free for research in place of the clinical courses ordinarily required. An average of 6 to 8 members of each class have taken advantage of this opportunity. Most of these then write theses based on their work. If the thesis is acceptable and the student passes an oral examination on both the thesis and the general field of which it is a part, he is granted the M.D. with honors in a special field. A number of the theses submitted have been fully equal to a Ph.D. thesis.

A considerably expanded tutorial program was instituted this year for the members of the first-year class. Enough tutors were appointed so that each group of 5 medical students would have its own tutor. These groups have been meeting weekly to discuss such things as the applications to research and to clinical medicine of the topics being discussed in the regular courses. This has generated wide interest in research; our chief problem is to make time available for the students to follow up their interest in research.

### The Rochester Student Fellowship Program

By *Leonard D. Fenninger.* University of Rochester School of Medicine and Dentistry.

Since 1926, The University of Rochester School of Medicine has offered full-year fellowships to students who have completed the first, second or third year of medical school. These fellowships have been an integral part of the educational program of the school.

Selection of candidates is based on their ability and scholarly potential. The fellow spends a full year in advanced study and research as a junior member of the department of his choice, in close association with senior members of the department. Each program is designed to give the fellow the greatest possible freedom for intellectual exploration.

Although student fellows may enroll in the graduate school of the university as candidates for advanced academic degrees, they are under no obligation to do so.

Fellowships have been awarded in all basic science departments and in the major clinical fields with the exception of surgery.

Although the field in which the fellowship has been taken has not, with the exception of pathology, influenced the ultimate field chosen following graduation, former fellows have entered full-time academic medicine and research more than twice as frequently as other graduates of this school. Thirty per cent of the former student fellows who have been out of school more than 5 years devote their time to teaching and research, while 12% of the graduates who did not have fellowships are full-time teachers and investigators.

Former student fellows have considered the fellowship the most valuable of their educational experience. It has awakened in them an interest in teaching and research and has served to establish close student-faculty relationships.

### The Honors Program of the New York University College of Medicine

By *Chandler A. Stetson, Jr.* New York University College of Medicine.

This program is in its second year of operation, and is designed to stimulate interest in and provide opportunity for undergraduate re-

search training. Participating students spend a half-day per week attending seminars and lectures on various problems in biological research; attend regular evening journal club meetings; have individual faculty advisors and abundant opportunities for independent laboratory research; are given an opportunity to take a fifth or "Medical Sciences" year of medical school, during which they spend full time at laboratory research; are encouraged to take courses in basic physical sciences or mathematics where indicated; and have opportunities for adjustment of the regular medical curriculum to provide blocks of 7 to 9 months of free time for research training. Selection of students for the program has been based primarily on the students' own interest, and participation has steadily increased until a quarter of the present first-year class is enrolled.

Although it is obviously too early to finally evaluate the program, it is the consensus of opinion among participating faculty and students that it has done much to further the graduate school atmosphere of the College of Medicine. Many of the invited lecturers have been biologists whose problems and approaches are ordinarily inaccessible to medical students, and this has made it possible for many students to recognize the medical sciences as intimately associated with and dependent on biology as a whole. Through participation in the journal clubs, students learn at the outset the importance and rewards of systematic and critical acquaintance with scientific periodical literature. Finally, the many opportunities afforded the students for participation at first hand in various laboratory research projects have resulted in a surprising number of first-rate efforts and accomplishments.

The long-range value of these opportunities must await further experience, but the reactions of the student body and faculty have thus far been enthusiastic and the program appears to have been of considerable benefit to both.

## ENDOCRINES AND METABOLISM

### Influence of Human Growth Hormone on Carbohydrate Metabolism in Man

By *Ernest J. Greenberg, Joseph Grayzel, Bronson S. Ray and Olof H. Pearson.* Sloan-Kettering Institute, New York.

Nondiabetic, hypophysectomized patients, maintained with replacement amounts of cortisone, have uniformly exhibited increased sensitivity to insulin. Previous observations on adrenalectomized patients maintained with similar amounts of cortisone had normal responses to insulin administration. Glucose tolerance tests after hypophysectomy are essentially normal. Hypophysectomy in diabetic patients consistently resulted in a decreased requirement for insulin to approximately 1/3 or less of the original insulin dosage. Hypophysectomy in patients with mild diabetes usually eliminated the requirement for insulin with a return of glucose tolerance to normal. Hypophysectomized, diabetic patients were maintained on cortisone (37.5 to 50 mg./day), this dosage being comparable to that used in nondiabetic, hypophysectomized patients. These observations indicate that hypophysectomy removes a factor which plays a significant role in the regulation of carbohydrate metabolism.

Human growth hormone (prepared by Raben) has been given in doses of 2.0 to 15 mg. daily intramuscularly to 5 hypophysectomized patients and one pituitary dwarf. In this dosage range, growth hormone produced either no change or a slight increase in the fasting and 2-hour postprandial blood sugar levels. In one nondiabetic hypophysectomized patient, 0.1 units of insulin per Kg. body weight given intravenously induced a 64% fall in blood sugar level after 20 minutes; after 9 days of growth hormone, the same amount of insulin induced a 20% fall in blood sugar level. In the pituitary dwarf, administration of growth hormone for 6 days altered the response to insulin in a similar manner. Human growth hormone in a dose of 5 mg./day was given on 2 separate occasions to an hypophysectomized diabetic patient who was being maintained on constant amounts of insulin, cortisone and triiodothyronine. Ketosis producing metabolic acidosis developed within 24 to 48 hours of administration of growth hormone on both occasions. A significant rise in fasting blood sugar and urinary glucose excretion was observed during one period of growth hormone administration. Ketosis subsided when growth hormone was withdrawn and insulin dosage was

temporarily increased. These observations indicate that human growth hormone induces diabetogenic effects in man.

**The Relative Influence of Maternal and Fetal Thyroid Function on Fetal and Postnatal Development**

By *Edward A. Carr, Jr., William H. Beierwaltes, Govind Raman and Norma R. Spafford.* Departments of Internal Medicine and Pharmacology, University of Michigan, Ann Arbor. (Aided by a grant for Research and Service in the Utilization of Human Resources from the State of Michigan.)

Maternal hypothyroidism is considered dangerous to the fetus. Bone x-rays suggest cretinism begins in utero. Yet labeled thyroxine studies have suggested maternal hormone cannot overcome fetal hypothyroidism.

We studied 15 mothers of cretins. Methods included history, physical examination, iodine$^{131}$ uptake, BMR, serum PBI (Barker's 1951 method) and cholesterol determinations. Fourteen proved euthyroid. One hypothyroid woman produced cretins in both former pregnancies. Now pregnant, her PBI, on 1400 mg. thyroid daily, is 15.3 (butanol extractable iodine, "BEI," 14.0) μg.%.

We measured iodine$^{131}$ uptake and/or PBI in 10 pregnant dogs and 10 litters. Mean pretreatment maternal PBI was 2.9 (S.E. ± 0.4). Two euthyroid mothers' litters' PBI's were 2.7 and 4.8. One euthyroid mother received iodine$^{131}$ one day antepartum. Her litter's mean thyroid: muscle iodine$^{131}$ concentration (T/M) ratio was 2250 (± 1240).

Four mothers were thyroidectomized. Their PBI's fell to 1.2 (mean). Their litters' PBI's were 2.3 (BEI, 0.5), 3.3, 3.8 and 5.2. The mother whose litter PBI was 2.3 had a PBI of 1.1 (BEI, 1.1). Two litters' T/M ratios were 2350 (± 810) and 3250 (± 1220). Several puppies of one hypothyroid mother showed intra-uterine underdevelopment. Another's litter grew poorly postnatally. A 5th thyroidectomized dog probably miscarried.

Three nonthyroidectomized pregnant dogs received thyroxine. Maternal PBI's reached 17.3 (BEI, 9.5, litter PBI 6.1, litter BEI, 2.7), 25 and 30 (litter PBI, 16). The first 2 litters' T/M ratios were 2760 (± 456) and 433 (± 289), the second suggesting fetal thyroid inhibition. Fetal serum counts showed adequate placental iodine$^{131}$ transport. Placental tissue concentrates iodine$^{131}$.

Maternal hypothyroidism does not depress fetal PBI or thyroid activity but otherwise endangers intra-uterine or postnatal development. Maternal euthyroidism does not prevent cretinism. Maternal exogenous hyperthyroidism may give puppies significant amounts of hormone. If the human placental threshold proves slightly below the canine, human cretinism may be preventable. This is under trial.

**The Effect of Thyroxine Loading on Protein-binding and Perpiheral Turnover of Thyroxine in Man**

By *John B. Richards, Norbert Freinkel and Sidney H. Ingbar.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, Department of Medicine, Harvard Medical School, Boston; and Howard Hughes Medical Institute.

In serum, virtually all thyroxine is protein-bound and during paper electrophoresis in barbital buffer is largely associated with a protein migrating in the inter-alpha area (thyroxine binding protein, TBP). It has been suggested that a small portion of thyroxine is unbound or "free," and that the rate of thyroxine turnover is a function of the concentration in serum of this "free" hormone. This hypothesis has been tested in 6 normal young individuals given blocking doses of tapazole. A tracer dose of radiothyroxine was administered intravenously and its rate of disappearance from the bloodstream determined. After 10 days, 4 mg. of stable thyroxine were rapidly infused and observations of disappearance of serum radioactivity were continued for one week. By paper electrophoresis in barbital buffer (pH 8.6) the distribution of radioactivity was assessed in sera obtained prior to and for several days following the thyroxine load. Loading with thyroxine was associated with elevation of serum PBI into the hyperthyroid range, marked displacement of radiothyroxine from TBP onto albumin, and profound increase in calculated values for free thyroxine. During the first 30 minutes after loading, serum radioactivity decreased markedly. However, the subsequent disappearance of radiothyroxine from the bloodstream did not differ appreciably from the control rate, despite persistent increase in PBI and displacement of thyroxine from TBP. Thus, after loading, calculated values of the thyroxine space were abrupt-

ly increased, although its fractional rate of turnover was unaffected. Hypermetabolism was not observed during the period of study.

In contrast, 3 subjects made hypermetabolic by prolonged administration of l-triiodothyronine displayed accelerated thyroxine turnover despite a reduction in PBI and in calculated values for "free" thyroxine.

The data suggest that factors other than the relative saturation of TBP or the absolute concentration of "free" thyroxine may govern the fractional rate of thyroxine turnover.

**Factors Influencing Thyrotropin Metabolism in the Rat Hypophysis: I. The Effect of Propylthiouracil (PTU)**

By *John L. Bakke and Nancy Lawrence.* V. A. Hospital and Department of Medicine, University of Washington School of Medicine, Seattle. (Aided by a grant of the Public Health Service.)

In a study of factors influencing thyrotropin metabolism in the rat hypophysis, frequent measurements of the changing thyrotropin concentration and content were made during PTU blockade. Female Wistar rats were placed on 0.05% PTU in the drinking water and killed in groups of 4–5 at 0, 2, 4, 8, 16, and 32 weeks. Blood was collected for subsequent TSH assay, the thyroid gland was weighed, and the hypophysis was weighed and assayed for TSH using the bovine thyroid slice assay method.

Thyroid weight increased steadily from 10.4 to 46.9 mg./100 Gm. body weight throughout the study. After 2 weeks this increase appeared to be linear on log-log coordinates. Pituitary weights declined from 6.48 mg./100 Gm. rat to 5.36 mg. after 2 weeks and then increased to 8.92 mg. after 32 weeks. The hypophyseal thyrotropin content fell from 7.55 to 1.68 U.S.P. milliunits/mg. after 2 weeks and 1.49 mu./mg. after 4 weeks, and then increased to 31.3 mu./mg. after 32 weeks. This increase was linear when log potency was plotted against log time. Thyrotropin content fell from 92.1 mu./rat to 16 mu./rat after 2 weeks and increased to 550 mu./rat after 32 weeks.

This diphasic potency curve offers an explanation for conflicting data reported in the past and reveals that hypophyseal thyrotropin content may fall markedly during a period when rapid release outstrips production capacity and may then rise to high levels in the face of a continued high release. Studies at 64 weeks and longer are in progress to establish maximum and to ascertain whether advanced myxedema is eventually associated with a decrease in hypophyseal thyrotropin.

**Factors Influencing Thyrotropin Metabolism in the Rat Hypophysis: II. The Effect of Triiodothyronine**

By *John L. Bakke and Nancy Lawrence.* V. A. Hospital and Department of Medicine, University of Washington School of Medicine, Seattle. (Aided by a grant of the Public Health Service.)

In a study of factors influencing thyrotropin metabolism in the rat hypophysis, frequent measurements of the changing thyrotropin concentration and content were made during triiodothyronine administration. Sixty female Sprague-Dawley rats were given 20 $\mu$g. of triiodothyronine daily and groups of 6 were killed at 0, 10, 30, 90 minutes, 4½ and 22 hours, and 4, 7, and 16 days. Control groups of 6 were killed at the start and end. Blood was collected for subsequent TSH assay, the thyroid gland was weighed, and the hypophysis was weighed and assayed for TSH using the bovine thyroid slice assay method. The hypophyseal weights did not change, but a significant ($p = < .01$) decline in thyroid weight occurred after 22 hours. After 16 days the 24 hour radioiodine uptake was 0.85%. Hypophyseal thyrotropin did not change until after the 4th day, when it fell from 11.0 to 0.9 U.S.P. milliunits/mg. In contrast to all other groups the intragroup individual variability of the 7th day group was high, some rats remaining at the control level, others falling to the low 0.9 mu. level of the 10 and 16-day groups.

Since thyroid metabolic activity is known to be inhibited within 5 hours, the constant hypophyseal thyrotropin level during the first 4 days suggests that hypophyseal thyrotropin production and release may remain closely balanced over a considerable range of declining hypophyseal release. An alternative explanation may invoke separate "metabolic" and "growth" TSH's, suggesting that this assay responds only to "growth TSH."

**Factors Influencing Thyrotropin Metabolism in the Rat Hypophysis: III. The Effect of Hypothalamic Homogenates**

By *John L. Bakke and Nancy Lawrence.* V. A.

Hospital and Department of Medicine, University of Washington School of Medicine, Seattle. (Aided by a grant of the Public Health Service.)

In a study of factors influencing thyrotropin metabolism in the rat hypophysis, the changing thyrotropin concentration and content was measured after injecting hypothalamic homogenates. Portions of anterior hypothalamus from the area associated with thyrotropin control in ablation experiments, and "control" material from the posterior hypothalamus and parietal cortex were removed from an anesthetized dog and frozen. These were homogenized and single intraperitoneal doses were injected into 21 "metabolically thyrodectomized" rats which had previously been made hypothyroid with PTU and a Remington diet, and then made euthyroid by the addition of a daily injection of 6 $\mu$g. dl-thyroxine for 7 days. Rats were killed at 0, 3, and 24 hours and blood was collected for subsequent TSH assay, the thyroid gland was weighed, and the hypophysis was weighed and assayed for thyrotropin using the bovine thyroid slice assay method. The anterior hypothalamic homogenate (183 mg. of tissue per dose) caused a fall from 7.80 U.S.P. milliunits/mg. (6.49–9.39; 95% fiducial limits) to 6.32 mu./mg. (5.14–7.79; 95% f.l.) after 3 hours; and to 6.47 mu./mg. (5.37–7.78; 95% f.l.) after 24 hours. Parietal cortex homogenate (504 mg.) caused a fall to 3.59 mu./mg. at 3 hours; 4.53 mu./mg. at 24 hours. In marked contrast, the "inactive" posterior hypothalamic homogenate (159 mg.) caused a significant rise to 10.16 mu./mg. (8.45–12.24; 95% f.l.) at 3 hours and then a fall to 2.62 mu./mg. (2.12–3.22; 95% f.l.) after 24 hours. Thyroid and hypophyseal weights did not change significantly during the experiment except 3 hours after the posterior hypothalamic homogenate, when a significant ($p < 0.02$) fall in thyroid weight occurred.

These results suggest that the posterior hypothalamic homogenate may contain a potent humoral agent which inhibited thyrotropin release prior to inhibiting synthesis. Further studies are needed to exclude other possibilities. Parallel studies with starvation, DNP, pitressin, hydrocortisone and estradiol failed to cause significant changes in thyrotropin content.

## The Occurrence of Circulating Triiodothyronine

By *Robert E. Mack and Kathleen T. Hart.* Radioisotope Laboratory, V. A. Hospital, St. Louis.

The present study was initiated to investigate the labeled hormone pattern in patients receiving radioiodine therapy. Serum was obtained 24 to 72 hours following $I^{131}$ administration. Separation of thyroxine and triiodothyronine was effected by filter paper chromatography in a butanol-dioxane-ammonia system. Eighty-five % of the labeled activity was at the triiodothyronine level in serum of a 48-year-old male having thyroid carcinoma with metastases at the 3rd thoracic and 4th lumbar vertebra. This thyroidectomized patient was clinically euthyroid with a PBI of 4.1 mg.% and an $I^{131}$ conversion ratio of 82%. Chromatograms of serum obtained at 24, 48 and 72 hours after a 2nd therapeutic dose of $I^{131}$ some 10 months later demonstrated approximately equal quantities of triiodothyronine and thyroxine in all 3 samples. Two other thyroidectomized patients whose underlying diagnosis was carcinoma of the thyroid were found to have labeled serum hormone only at the triiodothyronine level. One of these patients had $I^{131}$ concentrating metastatic lesions at the perihilar area of the lungs. The 3rd patient had a small remnant of thyroidal tissue at the level of the thyroid cartilage. At the time of her $I^{131}$ therapy, desiccated thyroid had been omitted for one month. In addition, TSH was administered for 4 days immediately prior to the therapeutic dose.

In the chromatographic system utilized, triiodothyronine cannot easily be distinguished from diiodothyronine. In previous studies, the appearance of serum triiodothyronine has been associated with thyroidal hyperplasia and rapid $I^{131}$ turnover. Such evidence supports the concept that the thyroid hormone anomaly reported here is probably triiodothyronine.

## Dissociation of the Effects of Uncoupled Oxidative Phosphorylation from other Actions of Triiodothyronine

By *William R. Beisel, Frank K. Austen and Milton E. Rubini.* Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C.

Uncoupling of oxidative phosphorylation may be responsible for the increased excretion of nitrogen and phosphorus in hyperthyroidism. By comparing the actions of 3,5,3' L-Triiodothyronine ($T_3$) to those of other uncoupling agents not possessing thyromimetic activity in other respects, it seemed possible to separate the effects of $T_3$ into 2 groups—effects apparently attribut-

able to uncoupling, and effects not attributable solely to uncoupling.

Using a metabolic balance technic, subjects were observed during $T_3$ (1.0 mg./day) or sodium salicylate (8.1 Gm./day) administration. Both drugs produced a similar rise in oxygen consumption and increased phosphorus excretion within the first day. $T_3$ caused a gradually increasing nitrogen excretion after 24 hours, agitation, tremor and tachycardia. In contrast, salicylate caused a much smaller increase in nitrogen excretion which did not begin for 4–8 days and disappeared immediately on stopping the drug; somatic manifestations were lacking. In a hypothyroid patient, salicylate also caused a prompt rise in oxygen consumption and phosphorus excretion, with a delayed moderate increase in nitrogen excretion. The cumulative loss of phosphorus in all patients was much greater than that attributable to tissue catabolism or bone dissolution.

Like $T_3$, various uncoupling agents (sodium salicylate, 2,4 dinitrophenol, and orthomercaptobenzoate) administered intravenously to dogs in acute experiments produced prompt phosphaturesis. The similarity of phosphaturetic action of these drugs to $T_3$ suggests a common mechanism of action, independent of thyromimetic activity.

These findings are compatible with two conclusions: (1) prompt phosphaturesis after $T_3$ like the rise in oxygen consumption may represent in vivo manifestations of an intracellular uncoupling of oxidative phosphorylation; and (2) the earlier and greater effect of $T_3$ on nitrogen balance compared to salicylate suggests that uncoupling may not be the only manner whereby $T_3$ induces a negative nitrogen balance.

### The Inhibition of Thyroid Hormone Production by Histidine

By *Mortimer S. Greenberg and Helena Wong.* Medical Service, Lemuel Shattuck Hospital, and Department of Medicine, Harvard Medical School. Boston. (Aided by a grant from the Arthritis and Metabolic Diseases Institute, Public Health Service.)

The action of certain drugs, structurally related to histidine, as metabolic antagonists to this essential amino acid was investigated in a microbiologic system (L. mesenteroides) requiring histidine for growth. Three antithyroid drugs, 1-methyl-2-mercapto-imidazole (methimazole), thiourea and propylthiouracil, were studied and all exhibited metabolic antagonism to histidine.

The role of histidine in the production of thyroid hormone was therefore studied (1) in vitro, by measuring its effect upon the incorporation of $I^{131}$ into the trichloroacetic acid (TCA) precipitable fraction of slices of surviving sheep thyroid gland suspended in Krebs-Ringer-phosphate solution, and (2) in vivo, by determining its effect upon the 24-hour uptake of $I^{131}$ by rat thyroid gland. In both the in vitro and the in vivo experiments the effect of histidine alone was studied as well as the effect of histidine upon the known block in organic binding of $I^{131}$ caused by methimazole. In the in vitro experiments both substances were used in concentrations which do not depress the uptake of $I^{131}$ by the thyroid slices.

Histidine did not inhibit the known effects of methimazole either in vivo or in vitro. Instead, histidine present in the suspending medium of the sheep thyroid slices in a concentration of $5 \times 10^{-2}$ M blocked the incorporation of $I^{131}$ into the TCA precipitable fraction in the absence of methimazole. Also, the injection of 200 to 400 mg. of histidine intraperitoneally into rats on a Purina Chow ration depressed moderately the 24-hour thyroidal uptake of $I^{131}$.

It is concluded that the activity of the antithyroid agents as metabolic antagonists to histidine in a bacterial species does not explain their effects upon thyroid physiology. Instead, it appears that histidine, structurally similar to methimazole, blocks the incorporation of iodide into thyroid hormone.

### Studies on Carbohydrate Metabolism in Hyperthyroidism

By *Paola I. Marchi, Albert E. Renold and Herbert A. Selenkow.* Departments of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, Boston. (Aided by a grant from the John A. Hartford Foundation, Inc., New York.)

The observation of glycosuria and diminished tolerance to carbohydrate feeding in hyperthyroid subjects has not been satisfactorily explained. It is generally assumed that the abnormally elevated and prolonged blood glucose levels following ingestion of a glucose load in hyperthyroid subjects results from an increased rate of intestinal absorption. However, other explanations for these observations have not been excluded. Intravenous glucose, fructose and galactose infusions of 10 minutes duration were performed on hyperthyroid subjects who repeatedly exhibited

fasting blood sugar levels below 100 mg.%. Serial blood samples were collected at 10-minute intervals for an 80-minute period after glucose infusion and for a 60-minute period after fructose and galactose. Blood glucose was determined by the Somogyi-Nelson method; blood fructose and galactose by the glucose oxidase technic.

Mean values plus or minus standard error of the fasting and serial postinfusion blood glucose levels in hyperthyroid patients were: 83±3, 285±20, 239±13, 206±13, 186±13, 166±13, 152±13, 141±13, 133±14. Comparable values for normal subjects were: 69±5, 257±25, 169±16, 129±10, 100±15, 78±9, 65±10, 55±8, 57±5. Thus, intravenous glucose tolerance in the hyperthyroid subjects was abnormal. In contrast, the rates of fructose and galactose disappearance were consistently found to be normal, although the resultant blood glucose levels following fructose and galactose infusions reached higher levels than in normal subjects. It appears that in hyperthyroidism the liver is able to metabolize fructose and galactose at a normal rate, but the glucose response differs from normal.

These results suggest that the carbohydrate abnormality observed in hyperthyroid subjects is not limited to increased intestinal absorption, but that there is also an intolerance to intravenously administered glucose. The abnormal rise in blood glucose following fructose and galactose infusions suggests that increased hepatic gluconeogenesis is, at least in part, responsible for these findings.

### Lung Function in Patients with Myxedema

By *William R. Wilson and George N. Bedell.* Pulmonary Research Laboratory, Department of Internal Medicine, College of Medicine, State University of Iowa. (Aided by a grant from the Iowa Tuberculosis and Health Association.)

The purpose of this paper is to report a study of lung function in patients with myxedema. Nine patients with clinical myxedema were studied. Laboratory evidence of myxedema included the following tests: basal metabolism −44 to +4% (mean = −17%); protein-bound iodine, 0.7 to 3.4 μg./100 ml. (mean = 2.1 μg./100 ml.); and $I_{131}$ uptake at 24 hours, 0 to 6% (mean = 2%). Pulmonary function studies were done using standard methods previously reported from this laboratory.

Six patients had normal arterial oxygen saturation and $P_{CO_2}$. In these patients the mean values of pulmonary function tests are listed as follows (%PN equals % of predicted normal): vital capacity, 77%PN; residual volume, 74%PN; total lung capacity, 74%PN; maximal breathing capacity (MBC), 64%PN; maximal expiratory flow rate (MEFR), 179 L./min.; maximal inspiratory flow rate (MIFR), 138 L./min.; diffusing capacity ($D_{L_{CO}}$), 14 ml./min./mm. Hg; and hematocrit, 33%.

Three patients had alveolar hypoventilation manifested by arterial hypoxemia (79.6, 82.2 and 90.6%) and elevated arterial $P_{CO_2}$ (49, 54 and 60 mm. Hg). In these patients the mean values of pulmonary function tests are listed as follows: vital capacity, 60%PN; residual volume, 41%PN; total lung capacity, 58%PN; MBC, 62%PN; MEFR, 152 L./min.; MIFR, 88 L./min.; $D_{L_{CO}}$, 15 ml./min./mm. Hg; and hematocrit, 40%. All 3 patients with alveolar hypoventilation were obese (388, 318, 277 pounds).

We conclude that myxedema produces reduced lung volumes, reduced mechanical efficiency and reduction in the diffusing capacity of the lungs. The reduction in diffusing capacity may be caused in part by anemia, but reduction of the total surface available for diffusion and perhaps increased thickness of the alveolar capillary membrane contribute significantly. When myxedema was associated with obesity, alveolar hypoventilation was found. Restudy of 2 of these 3 patients following treatment with dessicated thyroid and reducing diets demonstrated improvement in lung function.

### The Acute Effects of Parathyroid Extract in Patients with Diminished Renal Function and with Parathyroid Disease

By *Herbert Gershberg, Daniel R. Shields and Sally S. Kove.* Departments of Medicine and Physiology, New York University, College of Medicine, New York.

Parathyroid extract (PTE) was injected intravenously in 40 patients, including 15 with edema, ascites and diminished renal function. The glomerular filtration rate, renal plasma flow, phosphate reabsorption (% of filtered load), and sodium and potassium excretion were measured every 15 minutes for 90 minutes. The renal plasma flow increased immediately in every case, 15 to 100%. The phosphate reabsorption decreased 16% (7 to 40%) from an average value of 87% within 15–45 minutes. The filtration rate did not change consistently. The decrease in phosphate reabsorption

was independent of the effect on filtration rate, indicating a direct effect of PTE on tubular reabsorption of phosphate. In a patient with pseudohypoparathyroidism, PTE had no effect on phosphate reabsorption, though the renal plasma flow increased 40%.

In patients with filtration rates greater than 50 cc./min., phosphate reabsorption was not related to filtration rate. However, with filtration rates below 49 cc./min. phosphate reabsorption was depressed (47–74%). In the latter patients, injection of PTE decreased phosphate reabsorption an average of 36%, and in 3 of these, 46, 47 and 52%. When PTE was injected in a hyperparathyroid patient with a filtration rate of 48 cc./min., no change in phosphate reabsorption occurred, suggesting that the patient was under the influence of excessive endogenous parathyroid hormone and was resistant to the exogenous extract.

From these experiments several conclusions can be drawn: (1) that PTE has a direct effect on phosphate reabsorption; (2) that the acute response to PTE is of value in distinguishing hyperparathyroidism in the presence of reduced renal function; (3) that patients with reduced renal function do not necessarily have functional secondary hyperparathyroidism (as suggested by normal or enhanced response to PTE).

### Comparison of Methods for Evaluating Renal Excretion of Phosphate in Hyperparathyroidism

By *T. Franklin Williams, Joseph L. DeWalt, Robert W. Winters, Walter Hollander, Jr., Louis G. Welt and Charles H. Burnett.* Department of Medicine, University of North Carolina, Chapel Hill. (Aided by a grant from the United Medical Research Foundation of North Carolina.)

Of various methods used to describe the effects of parathyroid hormone upon renal excretion of phosphate, the measurement of maximal tubular reabsorption of phosphate (TmP), since it is independent within wide limits of the serum phosphate concentration, should be more closely correlated with parathyroid activity than either the percentage of filtered phosphate that is reabsorbed (%TRP) or the clearance of phosphate ($C_{PO_4}$). Inasmuch as the latter two are dependent upon serum phosphate concentration and thus upon phosphate load, they may be misleading as indices of parathyroid activity.

These possibilities have been evaluated in 7 patients with proven hyperparathyroidism by estimation of glomerular filtration rate (GFR) with inulin clearance, TmP, %TRP, and $C_{PO_4}$ before, and in 4 patients after, surgical treatment. TmP was measured at constant, elevated concentrations of phosphate in serum provided by loading and sustaining infusions of a solution of $Na_2HPO_4NaH_2PO_4$ (0.10 M $PO_4$, pH 7.4). Preoperatively TmP was always reduced: range, 33–103 $\mu$M/100 ml. GFR (normal range 121–150 $\mu$M/100 ml. GFR); %TRP, measured in 5 patients, was depressed in 3 (50–78%) but normal in 2 (91 and 95%); $C_{PO_4}$, measured in 5 patients, was elevated in 3 (20–23 ml./min.) but normal in 2 (7 and 10 ml./min.). The normal values of %TRP and $C_{PO_4}$ were obtained in patients on low-calcium, low-phosphate diets, a common situation in the evaluation of possible hyperparathyroidism. One week to 10 months postoperatively, TmP, %TRP and $C_{PO_4}$ had returned to or nearly to normal. GFR rose slightly in one patient and did not change in the others.

Thus the TmP appears to reflect satisfactorily the effects of endogenous parathyroid hormone upon renal tubular excretion of phosphate and is probably more dependable than the other methods evaluated. These patients showed no relation between parathyroid activity and GFR.

### The Effect of Sodium Phytate on Urinary and Serum Calcium

By *Harris D. Riley, Jr.* Department of Pediatrics and Poliomyelitis Respiratory and Rehabilitation Center, Vanderbilt University School of Medicine, Nashville.

Immobilization, if it persists for any period of time, results in hypercalciuria and eventual osteoporosis. Poliomyelitic quadriplegia represents in most cases a severe form of immobilization. Urolithiasis and associated complications are major obstacles in the rehabilitation and ultimate prognosis of these patients. Although stasis and infection are undoubtedly important factors in the development of urinary calculi in such patients, the hypercalciuria itself is a major factor.

Serial determinations of urinary calcium over a period of several weeks were made in 7 patients extensively paralyzed following poliomyelitis. Urinary creatinine, serum calcium, phosphorus and phosphatase were also measured. After an adequate control period during which the urinary calcium had stabilized, the administration of oral sodium phytate was begun. The effect of various treatment programs including different dose schedules, duration of therapy, and repeat courses

of treatment were studied. The effect of sodium phytate on iron metabolism was also studied.

In most cases the urinary calcium levels were normal early in the course of poliomyelitis and rose rapidly in several weeks to levels 3 to 4 times normal. On sodium phytate therapy the urinary calcium excretions fell at a variable rate but in most cases quite rapidly. When the drug was discontinued the excretion rose immediately and remained high until the drug was again reinstituted. The fall in calcium during repeat courses of sodium phytate was even more abrupt than during the initial course. Most of the patients exhibited a slight increase in serum calcium and phosphorus and a decrease in alkaline phosphatase while receiving sodium phytate. The relation of these findings to changes in the completeness of immobilization was investigated.

The results of these studies indicate that sodium phytate may markedly decrease the urinary excretion of calcium. This is accomplished by a combination of phytate and calcium in the intestinal tract to form insoluble and unabsorbable complexes.

### Clinical Study of an Adult with Hypophosphatasia

By *John A. Owen, Jr. and Herman Peskin.* Department of Internal Medicine, Medical College of Georgia, Augusta.

This report summarizes metabolic and genetic studies in progress in the case of a 30-year-old white woman hospitalized recently for pathologic fracture, apparently her first. Skeletal deformities, premature loss of deciduous teeth, delayed development, and considerable dwarfing were noted in childhood. As an adult she had generalized convulsions twice. Bilateral oophorectomy had been performed in 1948.

The patient was 4-2/12 feet tall, intelligent and cooperative. Joint mobility was limited by sinuous bowing of all the distal long bones and anterolateral femoral bowing. Deafness and blue sclerae were absent in the patient and her family. Fracture of the right femur was present.

Laboratory results were normal for: serum calcium, phosphorus, electrolytes, urinary calcium, phosphorus, and 17-ketosteroids, renal function tests and tubular reabsorption of phosphate. Alkaline phosphatase was 2.0 King-Armstrong units prior to iliac graft at the fracture site; subsequently it rose to 4.0 units and in 3 months fell to 2.0 again. Chromatography of the urine revealed phosphorylethanolamine in definitely abnormal amounts.

X-rays showed: (1) bowing of both ulnae, radii, tibiae, fibulae, and femora (coxa vara bilaterally); (2) pseudo-fractures of left scapula, both ilia, right ischium, left femur, and right fourth metatarsal; (3) fracture of right femur and old rib fractures, and (4) poor bone mineralization generally. Callus formed poorly postoperatively.

Bone biopsy showed fair callus formation without striking evidence of osteoid deficiency or excessive resorption.

X-rays made when the patient was 3½ years old are interpreted as being highly suggestive of infantile hypophosphatasia.

Although all members of the family are short, clinical bone disease is not evident in them. Studies of serum alkaline phosphatase have thus far given these results: father, 1.6; mother, 6.0 and 8.2; and brother, 1.8 King-Armstrong units.

It is believed that this is the first known example of hypophosphatasia in which the progression of clinical manifestations from infancy to adulthood is confirmed roentgenologically.

### Ketone Metabolism in Diabetes Mellitus

By *J. H. Darragh.* McGill University Clinic and Montreal General Hospital. (Aided by a grant from the Vineberg Fund.)

One of the metabolic defects in diabetes mellitus is a tendency to develop an increased concentration of ketone bodies in the blood, which may proceed to a severe ketoacidosis. This is a variable feature among diabetic patients; some never develop a severe ketosis, and in those who do, the cause is not always apparent. To obtain more information about ketone metabolism in diabetes mellitus, and in an attempt to correlate ketone metabolism with the clinical type of diabetes, the insulin requirements, nutritional status, incidence of degenerative complications, and the presence of infection, the concentration of blood ketones has been determined in 10 diabetic patients after withholding insulin.

In the first group of 5 patients with maturity-onset diabetes, none had a history of ketoacidosis, but one patient with hemochromatosis had a recent acute onset of diabetes with mild ketonemia (15 mg.% total ketones, expressed as acetone). Insulin was stopped, and daily fasting blood samples were analyzed for glucose and total ketones. All developed hyperglycemia, and in 3 patients there was no change in the blood ketones after

5 days without insulin. Two patients, including the patient with hemochromatosis, developed a slight increase in blood ketones after 3 days (to 4 mg.%).

In the second group of 5 patients with growth-onset diabetes, all had a history of one or more episodes of ketoacidosis. No insulin was given for 24 hours prior to the test period. Breakfast was omitted, and blood samples obtained every hour during the morning. The usual insulin was given prior to the noon meal. In 2 patients the initial blood ketones were normal, and there was no increase. Two patients had a higher initial level (5 and 7 mg.%), but there was no increase. One of these patients had an infected toe, and the other had severe diabetic retinitis, nephropathy, and peripheral neuritis. In the 5th patient, who had been admitted to hospital during the previous week with moderately severe ketoacidosis, the blood ketones increased from 2 to 4 mg.% during the 4-hour period.

Further observations are planned, using longer periods of withdrawal of insulin, and a stimulus to increased ketone production such as high fat diet.

### Spontaneous and Insulin-Induced Resistance of Peripheral Tissues to Insulin in Diabetes

By *Reubin Andres and Kenneth L. Zierler.* Departments of Environmental Medicine and Medicine, Johns Hopkins University and Hospital, Baltimore. (Aided by grants from ONR, NIH and Muscular Dystrophy Associations.)

Effect of insulin on peripheral tissues has been assessed by constant injection of insulin into a brachial artery and measurement of ipsilateral forearm metabolism by the Fick principle. Injection of 0.0001 u./Kg. body wt./min. for 26 minutes produced no systemic effect (measured by change in arterial glucose concentration), but produced local effects quantitatively different in normals and diabetics (age at onset 33 to 61 years; no regular insulin for 18 hours prior to study; not ketotic). Forearm glucose uptake in the basal state was 0.73 ± 0.24 $\mu$M/min./100 ml. forearm in 7 normal subjects, and −0.04 ± 0.35 (S.E.M.) in 7 diabetics. Intra-arterial insulin infusion, to a concentration of $3 \times 10^{-4}$ u./ml. forearm plasma, produced, at peak effect, a 10-fold increase in glucose uptake in normals. Three diabetics who had received therapeutic insulin for 2 days or less (2 had never had insulin) showed only half as much glucose uptake in response to insulin as the normals. Three diabetics who had received therapeutic insulin for 6 to 19 months showed little or no response to insulin. One diabetic who had received 9 days of insulin therapy responded in an intermediate fashion. Thus there is probably decreased glucose uptake by muscle in diabetes, diabetics show spontaneous resistance to insulin, and prolonged administration of insulin increases insulin resistance. The sort of insulin resistance demonstrated in these patients must reside either in blood (and act rapidly prior to first circulation through the forearm) or in forearm tissues.

### Significance of the Secretion of Insulin into the Portal Circulation

By *Leonard L. Madison and Roger H. Unger.* Department of Internal Medicine, University of Texas Southwestern Medical School, and V. A. Hospital, Dallas. (Aided by a grant from the Upjohn Company.)

Endogenous insulin is secreted into the portal circulation, and consequently 100% traverses the liver. By contrast, exogenous insulin enters the systemic circulation and only a fraction of the administered dose perfuses the liver. The purpose of this study was to compare the metabolic effects of unlabeled glucagon-free insulin and the distribution of $I^{131}$-labeled insulin when injected intraportally and intravenously.

In 8 dogs the effects of insulin on the magnitude of hypoglycemia and on the magnitude of increase in A-V glucose difference were compared after both routes of administration.

Different metabolic effects resulted from the different routes of administration. The magnitude of the hypoglycemia was similar after both routes. However, the peripheral injection resulted in a significantly ($p = <.01$) greater A-V glucose difference (mean 7 mg.%) compared to the intraportal injection (mean 4.2 mg.%). This suggests a greater hepatic effect and a smaller peripheral effect of intraportally administered insulin.

Hepatic binding of $I^{131}$-labeled insulin after both routes of administration was compared in 196 rats. Peak hepatic binding was significantly ($p = <.01$) greater after endoportal administration which resulted in 51% of the dose being bound to the liver in a single circulation. By contrast, mean total hepatic binding after peripheral injection was only 27% of the administered dose.

The data suggest an important physiologic role for the endoportal secretion of insulin. The initial transhepatic passage of intraportally injected insulin results not only in a greater hepatic

binding, but also in an apparently greater hepatic effect compared to insulin injected into the systemic circulation.

### The Development of Diabetes Mellitus in Patients with Nondiabetic Glycosuria

By *Irving Paul Ackerman, Stefan S. Fajans and Jerome W. Conn.* Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor.

Patients, who exhibit glycosuria during glucose tolerance tests and yet demonstrate normal tolerance for carbohydrate are defined as having nondiabetic glycosuria. A divergence of opinion exists regarding the incidence of subsequent development of diabetes in such individuals. The present study was undertaken to determine whether or not nondiabetic glycosuria is a precursor of diabetes mellitus.

Among University Hospital records for the past 30 years there were 153 cases who satisfied our criteria for nondiabetic-glycosuria. The records indicated that 4 of these patients had developed overt diabetes; and that where a subsequent glucose tolerance test had been done in 8 more of these patients 5 were found to be diabetic. Between the diagnoses of nondiabetic glycosuria and diabetes, 3 months to 13 years had elapsed.

We were able to study an additional 19 of these 153 patients with both glucose tolerance tests and cortisone-glucose tolerance tests. All were free of diabetic symptoms. Twelve of the 19 were found to have diabetes mellitus. One to 22 years had elapsed since the diagnosis of the nondiabetic glycosuria had been made. Of the 7 patients who had normal glucose tolerance tests, 2 gave abnormal responses to the cortisone-glucose tolerance test.

Thus of 27 patients originally found to have nondiabetic glycosuria, 17 (63%), although asymptomatic, were found to be diabetic when retested at a later time. Four more patients of the original group had developed symptomatic diabetes. Of these 21 patients with diabetes, only 6 had originally shown glycosuria in the fasting state.

We believe that nondiabetic glycosuria is not a benign condition; that it is indicative of potential diabetes mellitus; and that such patients should be retested periodically for the presence of diabetes.

### The Effect of Tolbutamide on Glucose and Nitrogen Metabolism in the Totally Depancreatized Dog

By *Henry L. Wildberger and Henry T. Ricketts.* Department of Medicine, University of Chicago.

We have previously reported the reduction by tolbutamide of blood and urinary glucose in totally depancreatized dogs receiving small, suboptimal amounts of insulin. The present experiments were undertaken to determine whether this effect is attributable to potentiation of the injected insulin.

Mongrel dogs were completely depancreatized, kept in metabolism cages and maintained throughout the following procedures on a constant diet and 2 small doses of crystalline insulin daily. Observations were carried out during alternating control and experimental periods, each of 2 week's duration. Addition of 0.25 Gm. of tolbutamide twice daily by mouth resulted in a significant decrease of blood sugar and urinary glucose as described previously, but nitrogen excretion was not affected. Addition of 2 to 4 units of protamine zinc insulin instead of tolbutamide produced a diminution of blood and urinary glucose comparable to that given by tolbutamide and a distinct fall in nitrogen excretion.

Since under these conditions tolbutamide does not cause nitrogen retention, whereas extra insulin does, it is concluded that the oral drug does not act by potentiating the maintenance doses of injected insulin. It seems likely that the primary action of tolbutamide takes place in the liver, for whose functional integrity a certain minimum of insulin is necessary.

### Effects of 2-Deoxyglucose on Carbohydrate Metabolism in the Rat and on the Hypoglycemic Response to Tolbutamide (Orinase)

By *Josiah Brown.* Department of Medicine, University of California Medical Center, Los Angeles.

To study the mechanism of the hypoglycemic effects of tolbutamide with 2-deoxyglucose (2-DG), which blocks carbohydrate metabolism, it was first necessary to determine the effects of 2-DG in the intact rat.

Intravenous administration of 2-DG was followed by glucose or fructose using the $C_{14}$ sugar as a tracer. Blood sugars, liver and muscle glycogen and $C_{14}$ content were measured. Rates of combustion of tracer doses of $C_{14}$ glucose and

fructose in unanesthetized rats were determined for 2–3 hours by continuous measurement of the expired $C_{14}O_2$ in an ionization chamber.

Intravenous administration of 400 mg./Kg. of 2-DG resulted in no change in blood glucose for 90–120 minutes and then a sharp rise of 60–90%. This rise did not occur in the absence of the adrenal medulla. In demedullated rats, 2-DG reduced by 70% the combustion of tracer doses of both $C_{14}$ glucose and fructose to $C_{14}O_2$. Injection of a glucose load following 2-DG resulted in a rise in blood glucose to double the fasting concentration for 3 hours contrasted to a return to the fasting level by 2 hours if deoxyglucose is omitted. In the rats given deoxyglucose there was 66% increased deposition of the glucose in the liver as glycogen.

Intravenous administration of 50 mg./Kg. of tolbutamide is followed by a 50% drop in blood glucose at 90–120 minutes. In intact rats, this response can be modified or inhibited by 2-DG depending on time of administration, but in demedullated rats, 2-DG did not in any way modify the hypoglycemic response to tolbutamide.

Combustion of glucose and fructose but not deposition as liver glycogen are inhibited by 2-DG which appears to stimulate epinephrine release in the absence of hypoglycemia. Since 2-DG does not modify the hypoglycemic response to tolbutamide, it appears that this response is not due to increased combustion of glucose.

**Oral Hypoglycemic Agents and Control of Stable Diabetes Mellitus in the Older Patient**

By *W. James Kuhl, Jr., John F. Becker and Harry J. Miller.* Medical Service, V. A. Research Hospital, and Department of Medicine, Northwestern University Medical School, Chicago.

In order to assess the relative usefulness of the oral hypoglycemic agents (OHA) in the treatment of diabetes mellitus, 31 patients were selected for transfer to the metabolic ward. One patient had labile diabetes. Thirty patients had stable diabetes of relatively recent onset, were not controlled by diet on an open ward or had a low requirement for exogenous insulin, and had had the onset of their diabetes during middle or older age. All were placed on a fixed diet adjusted to prevent weight loss, and insulin discontinued (except for the 1 patient with labile diabetes) for a minimum of 2 weeks. Body weight and qualitative glucose excretion was obtained daily, and fasting blood glucose and 24-hour glucose excretion determined twice weekly. Hematologic, liver function and thyroidal function studies were made at selected intervals for evidence of toxicity. Tolbutamide was administered if 2 weeks of diet alone did not result in absence of glycosuria and a fasting blood glucose below 130 mg.%.

One patient with labile diabetes was not controlled with a reduced dose of insulin and added OHA, and was returned to insulin therapy alone. Two patients with stable diabetes were returned to insulin because of lack of control with OHA. Thus, 10% of the patients were returned to insulin therapy. Fifteen patients (48%) were controlled by diet alone. Thirteen patients (42%) responded to OHA, and 12 were maintained on OHA. One patient who responded to OHA was returned to insulin therapy following a second cerebrovascular occlusion.

Comparison of those patients controlled by diet with those controlled by diet and OHA revealed that mean height (5'8" vs. 5'8") and mean weight (163 vs. 167 lb.) were similar. Mean age of the OHA group was greater (59.5 vs. 55.4 yr.), and mean duration of known diabetes longer (72 vs. 9 mos.). 50% of the OHA group had been treated with insulin vs. 40% of the diet group, and mean amount was similar (22 vs. 26 units).

These results suggest that previous estimates of OHA-responsive patients are too large, and that approximately 50% of these patients can be controlled by diet alone.

**Metabolic Effects of Phenethylformadinyliminourea (DBI) in Normal Subjects and in Diabetic Patients**

By *Stefan S. Fajans, John A. Moorhouse, H. Doorenbos, Lawrence H. Louis and Jerome W. Conn.* Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor.

Oral administration of DBI lowers the blood sugar of diabetic patients, diabetic animals and normal animals. An attempt has been made to study possible mechanisms of action of this drug.

In normal people 100–400 mg. of DBI for 1–12 days fails to lower the blood sugar. It produces no significant changes in glucose tolerance, sensitivity to insulin, or glucagon-induced hyperglycemia. Metabolic balance studies reveal no influence of DBI upon excretion of nitrogen, potassium, 17-hydroxycorticoids, or 17-ketosteroids. However, after infusion of a sodium pyruvate load, DBI slows the rate of fall of blood pyruvate and lactate.

DBI has a distinct blood-sugar-lowering effect in both stable and unstable diabetics. Metabolic balance studies indicate that significant decreases in hyperglycemia are not accompanied by diminished excretion of nitrogen or urinary steroids. Again, DBI produces no changes in glucose tolerance or glucagon-induced hyperglycemia. After the administration of DBI significant increases in blood pyruvate and lactate are observed both in the fasting state and during glucose tolerance tests.

The results are consistent with the in vitro results of others which suggest that DBI produces hypoglycemia by increasing anaerobic glycolysis. In diabetic patients there is no evidence that inhibition of adrenal function, decreased gluconeogenesis, or inactivation of glucagon activity are contributing factors. In significant contrast to the findings in ainmals, we observe no blood-sugar-decreasing effect of DBI in normal people.

### Clinical Studies with DBI

By *Thomas G. Skillman, Fred A. Kruger, Loren G. Peterson and George J. Hamwi.* Ohio State University, Department of Medicine, Division of Endocrinology and Metabolism, Columbus.

Twenty-four selected but uncontrolled hospitalized diabetics were given 150 to 400 mg. doses of DBI (phenethyldiguanide) daily for 6 to 100 days. Severity of diabetes and control values for thyroid, adrenal, hepatic, renal and hematopoetic function were established by a pretreatment period employing constant diet and insulin therapy. Patients demonstrating normoglycemia and aglycosuria with DBI therapy alone were considered responsive. Fifty-four % responded to a mean dose of 200 mg. DBI daily after 2–5 days. Overweight individuals, with less than 5 years' duration of diabetes, who took under 25 units of insulin and who failed to develop ketosis with insulin withdrawal, characterized those responding. A patient with Cushing's syndrome responded, while an hypophysectomized juvenile diabetic and a totally pancreatectomized subject did not. BMR, PBI, RAI, 17 OHCS excretion, liver function tests, hemograms and renal function were unaltered by DBI therapy. Additional studies suggest that DBI is ineffective as a sole agent in the total diabetic, but may stabilize the "brittle" diabetic when added to an insulin-diet regimen. Other analyses indicate that DBI alters intermediary pathways elevating blood lactic acid values (venous, arterial) and suppresses epinephrine and glucagon-induced glycogenolysis.

### A New Look at the Nutritional Program in Diabetes Mellitus

By *B. E. Lowenstein and Robin Lowenstein.* Metabolic Service, Department of Medicine, Dade County Hospital, Kendall, Florida.

Treatment of diabetes mellitus using conventional diets high in fat but limited in carbohydrate (and therefore limited in protein, a carbohydrate precursor) is physiologically irrational and therapeutically archaic.

The modern diet for diabetes mellitus is based on sound nutrition and is, moreover, corrective for the particular metabolic deficiencies of the disorder. Such a diet will fulfill the following criteria: (1) protein, not less than 120 Gm. per day; (2) fat, not more than 50 Gm. per day; (3) carbohydrate, at least 150 Gm. per day, plus whatever additional is necessary to meet the energy requirements of the individual patient. Fruit and honey are emphasized because of the fructose content; (4) dietary supplements: optimal amounts of vitamin B complex and the equivalent of the raw pancreas protective against fatty degeneration in diabetic dogs. These supplements were supplied by brewers' yeast and Entozyme tablets.

Such a diet has been in use as the heart of a therapeutic program for the treatment of diabetes mellitus at the Dade County Hospital at Kendall for the past 3½ years. More than 300 patients have been so treated. The following results have been observed. (1) Low mortality, only 2 deaths in the entire group. (2) Decreased morbidity as evidenced by fewer hospital admissions and fewer hospital in-patient days. No case of insulin shock or acidosis severe enough to warrant hospitalization. (3) No diabetic neuritis. (4) Fewer vascular complications with no amputations and improvement in diabetic retinitis when present. (5) No serious infections and few minor infections. (6) Decrease of tbc serum cholesterol to normal levels in *all* cases. (7) Extraordinary decrease in the insulin requirements, the insulin used after 3½ years being less than 1/10th of the amount required at the start of the study. (8) Notwithstanding the much lower doses of insulin used, improvement in the ability to utilize carbohydrate as evidenced by the glucose "tolerance" test curves.

### Evidence for Integrity of Hypothalamic-Pituitary-Adrenal System after Steroid Withdrawal

By *Thomas T. Amatruda, Jr., Dorothy Hollings-*

*worth, Nicholas D'Esopo, G. Virginia Upton and Philip K. Bondy.* V. A. Hospital, West Haven, and Department of Internal Medicine, Yale University School of Medicine, New Haven.

The development of fever, anorexia, myalgia, arthralgia and desquammation after withdrawal of steroid therapy has been attributed to hypoadrenalism. In some cases the hypoadrenal state is alleged to persist for months. This "hypoadrenal" syndrome uniformly occurred in 8 tuberculous patients with hyperadrenocorticism produced by prednisone (30 mg. daily) and zinc ACTH (40 units every other day) for 6 months. They also received antituberculous therapy. As previously reported, these patients exhibited a normal plasma cortisol response to ACTH while having symptoms.

By utilizing insulin hypoglycemia the hypothalamic-pituitary-adrenal system was investigated in these patients while they were symptomatic or shortly thereafter. Seven patients and 9 tuberculous controls received 0.15 unit/Kg. of glucagon-free insulin i.v. Blood glucose was determined (Somogyi-Nelson) at 30-minute intervals for 3 hours, and plasma cortisol (Bondy) was measured at 1 and 2 hours. Surprisingly, the patients experienced little or no clinical hypoglycemia symptoms, although 7 controls had moderate to severe reactions. The decreased sensitivity to insulin was also evident in the higher blood glucose concentration at 30 minutes, 48.3 ± 4.9 mg.% (mean ± S.E.) compared to the controls, 34.0 ± 3.1 mg.% ($P<.05$). There were no significant differences at the other times. Plasma cortisol concentrations at 0, 60 and 120 minutes were 10.5 ± 2.7, 18.9 ± 2.2 and 17.3 ± 2.3 μg.% in the patients, compared to 12.6 ± 0.9, 20.6 ± 1.4 and 19.3 ± 2.1 in the controls. No significant differences were found between the groups. One patient uneventfully tolerated pulmonary lobectomy without supporting steroid therapy. His plasma cortisol levels rose from 7.3 to 28.1 μg.% postoperatively, a response comparable to that of 5 control lobectomy patients.

These patients exhibited normal hypoglycemia responsiveness suggesting that their "steroid withdrawal syndrome" was not due to dysfunction of the hypothalamic-pituitary-adrenal system. The true nature of the syndrome remains to be elucidated.

## Conversion of Corticosterone to Aldosterone in Normal Man

By *Holbrooke S. Seltzer and Dale A. Clark.* Departments of Medicine and Biochemistry, University of Texas Southwestern Medical School, and V. A. Hospital, Dallas and McKinney, Texas.

Although corticosterone is a possible biosynthetic precursor of aldosterone, in vitro experiments have shown quantitatively insignificant conversion of the former steroid to the latter. The present study disclosed sharply increased urinary aldosterone excretion during prolonged administration of corticosterone to normal man.

Continuous infusions of corticosterone were maintained intravenously for 48 hours in 3 normal males. Daily dosage was 200 mg. in 1 subject and 300 mg. in the others. Urine was assayed for electrolytes, 17-hydroxycorticoids and aldosterone (physico-chemical method of Neher-Wettstein).

*Aldosterone:* Both subjects who received 300 mg. corticosterone daily showed markedly increased aldosterone output the first infusion day (11.8 to 21.3 μg. and 24.1 to 84.7 μg., respectively). On the 2nd infusion day, however, excretion *fell* to 15.6 and 42.5 μg. During the post-infusion period, decreasing aldosterone titers reached sub-baseline levels (7.2 and 13.5 μg.) on the 2nd day, then rebounded to normal. The subject given 200 mg. corticosterone daily had a flatter but qualitatively similar excretory pattern.

*17-hydroxycorticoids:* Corticosterone administration depressed average 17-hydroxycorticoid excretion for the group from 8.9 to 1.1 mg./day, with prompt rebound to normal the first post-infusion day.

*Sodium-potassium:* Baseline urinary Na/K ratio for all subjects averaged 1.17 ± 0.34 (S.D.). During corticosterone administration, sodium retention coupled with potassium loss reduced Na/K to 0.67 ± 0.27 ($p<0.025$). Profound post-infusion sodium diuresis and moderate potassium retention greatly increased Na/K to 4.40 ± 1.07 ($p<0.01$).

These preliminary data suggest, first, that mineralocorticoid effects of corticosterone may be mediated via in vivo C-18-oxidation of quantitatively tiny amounts to aldosterone. Secondly, high corticosterone dosage suppresses glucocorticoid output, and seems also to inhibit endogenous aldosterone secretion. Finally, the possibility that corticosterone is a physiologic intermediate in aldosterone biosynthesis remains unanswered, since extra-adrenal steroid conversion has not been excluded by this study.

### A Water Diuretic Effect of Mineralocorticoids in Hydrocortisone-treated Adrenal Insufficiency

By *Hieronymus Doorenbos, David H. P. Streeten and Jerome W. Conn.* Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor.

Patients with adrenal insufficiency (Addison's disease or after bilateral adrenalectomy) maintained on oral hydrocortisone exhibit a brisk water diuresis of about 2 hours duration when given a single dose of either 9-alpha-fluorohydrocortisone (1 mg.) or 2-methyl-9-alpha-fluorohydrocortisone (0.1 mg.).

For a 7-hour period all patients remained recumbent and were given no solid food. Constancy of hydration was approached by giving fluids orally, hourly. Completeness of urine collections was ensured by using indwelling catheters in female subjects, and in males by expressing the results in terms of creatinine excretion.

In 9 tests on 7 patients, a water diuresis was observed after a single intravenous injection of either of these mineralocorticoids. The peak of water diuresis occurred during the 2nd hour after the injection, hourly urine volumes increasing in some instances to 3 times the control volumes. The glomerular filtration rate did not increase during the time that water diuresis occurred. Studies with radioactive bromide disclosed no change in total extracellular fluid compartment.

No diuretic effect was observed with the same doses of these mineralocorticoids when hydrocortisone was withheld (4 tests in 3 patients).

It is suggested that the water diuresis brought about by administration of a mineralocorticoid to the hydrocortisone-treated Addisonian (or adrenalectomized individual) represents correction of an abnormality of water metabolism induced when hydrocortisone is the sole steroid used as replacement therapy. This activity of mineralocorticoids is independent of their effects upon electrolyte metabolism.

### Effects of Adrenalectomy and Adrenal Steroids on Extracellular Fluid and Bone Composition

By *A. B. Borle and G. Nichols, Jr.* Departments of Biochemistry and Medicine, Harvard Medical School, Boston. (Aided by a grant from the U. S. Public Health Service.)

Evidence from in vitro studies is available that sodium will exchange for calcium in synthetic hydroxyapatite. This laboratory has demonstrated, in vivo, an increased bone sodium in sodium-loaded rats and a decreased bone sodium in sodium-depleted animals. Moreover, decreased bone sodium has been found in adrenalectomized animals. On the other hand, Clark obtained a decreased radiocalcium uptake in bone following administration of adrenal steroids. The purpose of this experiment was to test the hypothesis that these heteroionic exchanges in bone may be controlled by the adrenals, specifically that Ca may replace Na at the bone crystal surface in adrenal insufficiency, while, under adrenal stimulus, the reverse may occur.

Three groups of rats were studied for 7 days. Group I was adrenalectomized; group II received 10 mg. Prednisolone daily; group III, control animals. Na, K, Ca, Cl, P were measured in plasma and bone. ECF values were calculated. In group I, ECF Na and Ca decreased 6.9% and 8.7% respectively, as compared with controls. In bone, Na decreased 5.6% (19 mEq./Kg. ash) and Ca increased 2.8% (428 mEq./Kg. ash). In group II, despite a 16.8% fall in ECF Ca, bone Ca rose 3.5% (558 mEq./Kg. ash), while Na remained at control levels.

Thus, although the results of group I confirmed our assumptions, the bone changes in group II went in a direction opposite to that expected. This suggests that the usual concept of bone-ECF equilibrium as a 2-phase system may be erroneous. Instead we postulate a 3-phase system in which equilibrium is controlled by local changes in the organic matrix.

### The Metabolic and ACTH-suppressing Activity of Certain $C_{21}$-deoxysteroids in Man

By *Mortimer B. Lipsett and Delbert M. Bergenstal.* Endocrinology Branch, National Cancer Institute, Bethesda.

Steroids with glucocorticoid activity in man have possessed a $C_{21}$-hydroxyl group, and glucocorticoid effects have not been seen when this functional group was absent. We wish to report that a $C_{21}$-deoxysteroid, $\Delta^{1,4}$ pregnene-9α-fluoro, 6 methyl, 11β, 17α, diol, -3,20-dione, was capable of causing a large negative nitrogen balance consistent with glucocorticoid activity.

At a dose of 15 mg./day of this compound, no detectable metabolic changes were observed in 2 patients studied by standard metabolic balance technics. Marked suppression of adrenal function was noted at this dose level. At 45 mg./day, sodium retention appeared and there was a slight negative nitrogen balance. When 100 mg./day was administered, the urinary nitrogen increased 6 Gm. on the 6th day. This large negative nitrogen balance was accompanied by a phosphate and potassium diuresis.

In order to assess the importance of the various functional groups, other $C_{21}$-deoxysteroids were tested for their ability to suppress adrenal function. At a dose level of 15 mg./day, $\Delta^1$, 9α-fluoro, 21-deoxyhydrocortisone; $\Delta^1$, 9α, 21-difluoro, 21-deoxyhydrocortisone, and 9α-fluoro, 21-deoxyhydrocortisone produced only a 50% decrease in urinary 17-hydroxy corticoids in contrast to the almost complete suppression observed with the 6-methyl derivative. These compounds also produced sodium retention.

A comparison of these compounds with the 6-methyl derivative reveals that methylation at $C_6$ greatly enhances the glucocorticoid and ACTH-suppressing activity of $C_{21}$-deoxysteroids. The data also suggest that methylation of $C_6$ inhibits the sodium-retaining effect of the 9α-fluoro group. Thus the $C_6$-methyl group is similar to the $\Delta^{1,2}$ function as it leads to simultaneous enhancement of glucocorticoid activity and to a decrease in sodium-retaining activity.

### The Effect of Therapeutic Doses of Salicylates on Adrenal Cortical Secretory Activity in Normal Subjects

By *Mattie C. Gautney, Alexander Ulloa, Howard L. Holley, Getrude B. Myer, Etherine Pearson and S. Richardson Hill, Jr.* Department of Medicine and University Hospital, Medical College of Alabama, and V. A. Hospital, Birmingham. (Aided by a grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.)

The similar therapeutic effect obtained from salicylates and glucosteroid therapy in rheumatic diseases has led to speculation that their mode of action is similar, possibly that salicylates increase adrenal cortical activity. Several studies have been undertaken to delineate this, but the reported results remain controversial. The present study is an attempt to determine whether salicylates in moderate therapeutic doses alter the 24-hour urinary excretion of adrenocortical metabolites.

A group of 10 healthy individuals, while performing their usual daily activities, were studied. There were 6 males and 4 females, with ages ranging from 23 to 50, with a mean of 37 years. Salicylates were given orally as aspirin in doses of 2.6 Gm. daily in 4 divided doses for a period of 4 days. Twenty-four hour urine specimens were collected from 7:00 a.m. to 7:00 a.m., for 2 days prior to the institution of salicylate therapy and on the last 2 days of treatmnet. Duplicate determinations of creatinine, 17-ketosteroids and 17-hydroxycorticosteroids were carried out on each specimen. All of the control and treatment urinary steroid values were within normal range, as obtained in our laboratory. There were no significant differences between the control and treatment urinary 17-ketosteroid levels which were 14.9 mg. before and 14.6 mg./24 hours during salicylate therapy. Similarly there was no significant difference between the mean urinary 17-hydroxycorticosteroid levels obtained during the control and treatment periods. The mean value for the pretreatment period was 5.8 mg. and for the treatment period 5.5 mg./24 hours.

It would appear that salicylate administration in this dose range does not consistently influence the urinary excretion of these two adrenal cortical metabolites.

### The Significance of Aldosterone Secretion during Dietary Sodium Deprivation in Normal Subjects

By *J. Crabbé, E. J. Ross and G. W. Thorn.* Department of Medicine, Harvard Medical School, Boston.

In normal individuals, the rise in urinary excretion of aldosterone on a low sodium intake is commonly believed to be secondary and proportional to the loss of extracellular fluid resulting from the sodium deficit, and to be quantitatively reflected by the ensuing reduction in urinary sodium.

An increased excretion of aldosterone in urine was indeed observed in 13 experiments performed on 8 normal males maintained on low sodium diets (less than 10 mEq. daily) for periods of 5–10 days. However, for a given sodium deficit, (1) this increase was highly variable in its time of onset and magnitude, when individuals were compared; (2) it was more pronounced in a subject when sodium withdrawal was achieved by sudden rather than progressive restriction, even more so when sudden restriction was combined with administration of acetazolamide plus potassium. Furthermore, the rise in urinary aldosterone generally did not progress until resumption of normal salt intake; except in two instances, the values for urinary aldosterone reached a peak followed by stabilization somewhere between this maximum and control values, despite continuing decrease in urinary sodium. Addition of salt to the diet resulted in its almost quantitative retention, whereas aldosterone immediately returned to normal levels.

Thus the pattern of aldosterone secretion in

response to sodium deprivation was not reflected in the degree of sodium retention, as expressed by the amounts excreted in urine. This indicates that mechanisms other than aldosterone are important in the normal adaptation to low sodium intake, unless some "permissive" role is attributed to aldosterone.

On the other hand, the accentuation of the sodium and weight deficits in the presence of a decreasing aldosterone excretion, during sodium restriction, makes it difficult to support the theory of a quantitative relationship existing between the extent of aldosterone response and that of the extracellular volume deficit.

### Alteration of Citrate Metabolism by Prednisone Therapy

By *Julius Mueller, Henry M. Lemon, Marcia Kelman, Joseph M. Looney and William H. Chasen.* Departments of Medicine and Biochemistry, Boston University School of Medicine, and Boston V. A. Out-Patient Clinic, Boston. (Aided by research grants from the United States Public Health Service.)

Citric acid constitutes over 1% of organic bone matrix where it may play a role in calcification. The metabolism of citrate has been explored in patients with and without bone disease by evaluating serum calcium, phosphorus, glucose and citrate concentrations after a 12-hour fast, and the response of these substances 5 min. and 30 min. following rapid i.v. injection of 0.50 Gm. sodium citrate. Ettinger's pentobromoacetone method for citrate analysis has been used. Thirty-five normal and arthritic females averaged 25% higher citrate concentration (37–42 μg./ml.) than 42 comparable males. The increase in citrate concentration at 5 min. is also about 30% greater in females. Assuming equilibration between plasma and extracellular fluid, citrate dilution occurs into 17 L. volume in males and 12 L. volume in females. From 5 min. to 30 min. citrate concentration almost returns to preinjection values in both sexes at a rate of 1 μg./ml./min., but no changes were observed in calcium, phosphorus or glucose. The net total clearance rate of citrate in this period averages 993 mg./hr. in males and 765 mg./hr. in females. In 64 patients with metastatic cancer, rheumatoid arthritis, and osteoarthritis not treated by steroids the clearance rate appeared fairly constant, but a 30–50% decrease occurred following chronic therapy with 10–30 mg. of prednisone daily. The fasting level of citrate also decreased following operations or prednisone therapy. Three patients with osteoporosis had clearance rates of 560–610 mg./hr. Hypercalcemia secondary to osteolytic metastases was always accompanied by fasting hypercitricemia, but the latter also occurred in metastatic carcinoma without detectable bone involvement or hypercalcemia. It is concluded that adrenal cortical hormones depress fasting citrate blood concentrations and citrate clearance rates from blood.

### Depression of Serum and Urinary Citric Acid by 17-Hydroxycorticosteroids

By *Dorothy H. Henneman and Philip H. Henneman.* Peter Bent Brigham and Massachusetts General Hospitals, Harvard Medical School.

To define better the biologic significance of citric acid, the influence of the blood level of 17-hydroxycorticosteroids on serum and urinary concentrations of citric acid has been studied in 16 patients on constant diets during complete balance studies. Increased blood levels of 17-hydroxycorticosteroids as in Cushing's syndrome, during ACTH or cortisone administration, or following trauma and surgery were associated with a depression of serum and urinary citric acid to levels significantly below normal. Decreased blood levels of these hormones following adrenalectomy for Cushing's syndrome or following cessation of steroid therapy were associated with a prompt rise in citric acid. The changes in urinary citric acid presumably reflect but do not produce the changes in serum citric acid concentrations. Decreases in citric acid occurred despite increased urinary calcium (as following ACTH, vitamin D and cortisone administration); an increase in citric acid occurred following adrenalectomy for Cushing's syndrome prior to any changes in calcium excretion. No gross changes in acid-base balance were detected in these studies and the direction of change in sodium balance which did occur would not explain the direction of change in citric acid. Changes in potassium balance could not account for either the early rise following adrenalectomy or the prompt and persistent fall in urinary citric acid during cortisone therapy. Thus the effects of 17-hydroxycorticosteroids on citric acid apparently are more potent than the previously reported influences of vitamin D or of changes in urinary calcium or potassium.

Study of intermediary carbohydrate metabolism in patients with Cushing's syndrome suggested that 17-hydroxycorticosteroids inhibit the conversion of pyruvic acid to acetyl-CoA. The

present studies further suggest that the decreased formation of acetyl-CoA may in turn result in a decreased formation of citric acid from the coupling of acetyl-CoA and oxaloacetic acid.

**Interrelationships between Adrenal Cortical and Medullary Secretory Activity in Patients with Pheochromocytoma**

By *Sidney B. Chenault, Mattie C. Gautney, Jean H. McNeil and S. Richardson Hill, Jr.* Department of Medicine and University Hospital, Medical College of Alabama, and V. A. Hospital, Birmingham. (Aided by a grant from the Alabama Division of the American Cancer Society and the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health.)

Investigations on the interrelationship between adrenal medullary and cortical secretory activity have yielded conflicting results.

The present investigation of 3 patients with pheochromocytoma was undertaken to compare the tumor content of epinephrine and norepinephrine with the adrenal cortical response to maximal stimulation by ACTH. In addition, an attempt was made to correlate the hormonal studies with the clinical syndrome. All patients had marked, sustained hypertension with episodes suggesting increased circulating epinephrine levels. One patient with hypermetabolism, diabetes, and total urinary catecholamine levels of 38 and 96 μg.% had elevated control levels of total urinary 17-hydroxycorticosteroids which increased abnormally following ACTH administration. Following removal of the tumor at the bifurcation of the aorta the clinical state returned to normal and there was a normal urinary steroid response to ACTH which was not increased further by simultaneous epinephrine and ACTH administration. There was no gross abnormality of either adrenal by palpation. The tumor contained 7.5 mg. of total catecholamines per Gm. of tumor, with greater than 6.2 mg. as norepinephrine.

Two other patients with carbohydrate intolerance, but not overt diabetes, had a normal urinary steroid response to ACTH administration. Total urinary catecholamine levels were elevated in both patients to 22 and 16u%. Assay of one patient's tumor revealed 0.55 mg. catecholamines per Gm. of tissue, predominantly norepinephrine.

These studies illustrate one patient with apparently normal adrenal cortices and with an extra-adrenal pheochromocytoma who had increased levels of urinary 17-hydroxycorticosteroids, responding abnormally to ACTH which returned to normal following removal of the tumor. The data from this patient suggest that the increased urinary corticoid levels observed prior to operation were not the result of an increased secretion of epinephrine by the tumor. These results also suggest that the clinical picture in these patients does not necessarily reflect the predominant type of adrenal medullary hormone secreted by the tumor.

**The Apparent Lack of Effect of Diphenylhydantoin (Dilantin) upon Adrenal Cortical Response to ACTH in Man**

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The study was undertaken in order to evaluate earlier reports describing alterations in structure and function of the adrenal cortex following prolonged administration of the anticonvulsant, diphenylhydantoin. Bray, Ely, and Kelley reported decreased response of plasma 17-OH-corticosteroid levels to intramuscular ACTH in 19 children with convulsive disorders treated with diphenylhydantoin, and speculated that an ancillary mechanism of the drug's anticonvulsant property might reside in its ability to produce a degree of adrenal unresponsiveness. To confirm this observation in adults, 15 patients (aged 14–74) with seizures of unknown etiology treated for 11 months to 10 years with 0.2–0.6 Gm./day diphenylhydantoin were subjected to 4-hour intravenous ACTH tests. Plasma 17-OH-corticosteroid levels were determined by the Silber-Porter method as modified in this laboratory and by Peterson et al. In 13 patients, steroid response to ACTH was normal, in 2 patients (in whom treatment was started before puberty), subnormal. Average response of the treated group (plasma 17-OH-corticosteroid level before ACTH, 20 μg. %, after ACTH, 48 μg. %) was similar to that observed in 40 normal subjects (level before ACTH, 17 μg. %, after ACTH, 46 μg. %). To exclude possible effects of interfering chromogen, a pool of post-ACTH plasma from 3 diphenylhydantoin-treated patients was analyzed by paper chromatography (Zaffaroni, chloroform:methanol-formamide), the cortisol spot was eluted and estimated by ultraviolet absorption. 93% of Porter-Silber chromogen was thus quantitated as corti-

sol. The discrepancy between the findings of this and the earlier study is not readily explained. However, the data of the present investigation suggest that any beneficial effect of diphenylhydantoin upon seizure threshold in adults cannot generally be ascribed to a relative suppression of adrenal cortical responsiveness.

### Human Bone Electrolytes in Various Disease States

By *Edmund D. Pellegrino and Saul J. Farber.* New York University College of Medicine, New York, and Hunterdon Medical Center, Flemington, New Jersey.

Animal experiments indicate that bone sodium participates in sodium metabolism in acidosis, and from these data extrapolations are made to disease states in man. Data on electrolyte concentrations in human bone in normals and in various diseases are scanty. The present study explores concentrations in human cortical bone in normals and some disease states.

Specimens of tibial and other cortical bone, removed at operation or autopsy, were ground to a fine powder. These were then heated to constant weight, ashed at 1200 F. for 24 hours and the residue dissolved in 3 N HCL. The solution was placed on Dowex 50 columns, and fractional eluates were analyzed for sodium, potassium, calcium and phosphorus. Fifty bones from 34 males and 16 females, in age range from a premature infant to 92 years, were analyzed. A wide spectrum of diseases was investigated. Bone specimens obtained at operation or from patients dead from trauma or acute myocardial infarction were considered normal.

The mean values for sodium in 15 normals were 248 mEq./Gm., Ca 11 mEq./Gm. and P was 114 mg./Gm. K concentration was usually below 10 mEq./Gm. No significant differences were noted between patients with cardiac disease, with and without edema, patients with malignancies, patients with renal disease and normals. No correlation existed between bone electrolytes and serum Na, Cl, BUN, or K. In 4 cases of severe metabolic acidosis due to uremia the mean value was 232 mEq./Gm. Na with no appreciable variation in Ca or P. In respiratory acidosis, the bone Na, Ca, P were normal.

It is concluded that the average values of the major electrolytes of tibial human bone are quite constant in a wide variety of diseases and independent of age, sex, serum levels of Na, K, Cl, BUN and $CO_2$, with the possible exception of severe metabolic acidosis.

### The Effect of Dilutional Hyponatremia on Bone Sodium

By *Robert W. Winters, Robert T. Whitlock and Louis G. Welt.* Departments of Medicine and Pediatrics, University of North Carolina School of Medicine, Chapel Hill.

Previous investigations of others have demonstrated that acidosis (both metabolic and respiratory), and salt depletion are accompanied by a reduction in the amount of sodium in bone. Some of these studies were complicated by hyponatremia which raised the question concerning the role of a reduction in the concentration of sodium in serum with respect to the disposition of sodium between bone and the extracellular fluid. This question was examined in the present study.

Three groups of fasting adult male rats received water loads by gavage at hourly intervals for 24 hours according to the following plan: group I, to maintain initial body weight constant; groups II and III, to provide a sustained increase of 5% and 15% of initial body weight respectively. All rats received vasopressin in oil at 0 and at 12 hours.

Rats of groups II and III developed profound hyponatremia (112 ± 10.4 and 98 ± 4.7 mEq./L., respectively) compared to group I (138 ± 8.1 mEq./L.). However, there were no significant changes in the bone sodium values among the 3 groups (I, 261.6 ± 11.3; II, 253.6 ± 6.1; III, 259 ± 7.2 mEq./Kg. fat-free dry bone).

Thus despite the induction of a striking dilutional hyponatremia and a larger gradient for sodium between bone and the extracellular fluid there was no significant transfer of sodium from bone.

### The Response of Tissue Electrolytes to Respiratory Acidosis

By *Howard Levitin, Carol R. Jockers and Franklin H. Epstein.* Department of Internal Medicine, Yale University School of Medicine, New Haven.

Although tissues of mucle and bone play an important role in buffering strong mineral acids, the response of these body buffers to carbonic acid has not been well delineated. The present

experiment was undertaken to examine the role of liver, muscle and bone in buffering respiratory acidosis.

Sprague–Dawley female rats weighing 175–225 Gm. were studied in 2 groups of 10 rats each. The experimental animals were placed in 8% $CO_2$ in air for 24 hours while the control animals were kept in room air. Food was removed from both groups at the beginning of the experiment. Immediately after removal from the $CO_2$ chamber, the animals were sacrificed by exsanguination from the abdominal aorta and specimens of liver, muscle and bone were obtained for analysis.

Exposure to 8% $CO_2$ caused an elevation of the plasma $CO_2$ content and a decre se in plasma Cl of 7 mEq./L. respectively. Plasma Na was unchanged while plasma K was slightly but significantly elevated.

There was no difference between the experimental and control group in the water and fat content of liver, muscle and bone. Tissue electrolytes were determined as mEq./100 Gm. of fat-free dry solids. The experimental and control groups showed no difference in the Na content of liver, muscle, or bone, nor any difference in mg. Ca/100 Gm. of dry bone. Muscle K of rats in $CO_2$ decreased significantly while bone K increased slightly and liver K remained similar to control values. These results differed from those obtained by previous workers, possibly because the short duration of the present experiment avoided certain metabolic effects which complicate long-term studies of experimental respiratory acidosis.

The data suggest that K proteinate of muscle is capable of buffering carbonic acid as well as stronger acids, but that the combination of Na with carbonate in bone, although capable of buffering "metabolic acidosis," cannot serve as an effective buffer for carbonic acid.

### Radioactive Magnesium ($Mg^{28}$) Metabolism in Man

By *Barnett Zumoff, E. H. Bernstein, J. J. Imarisio and Leon Hellman.* Sloan–Kettering Institute for Cancer Research, New York.

The recent discovery of a short-lived radioactive magnesium isotope ($Mg^{28}$) provides a tool for investigation of the kinetic behavior of magnesium in intact human subjects. The following observations have been made using $Mg^{28}Cl_2$: (1) From 30–85% of an oral dose was absorbed. Absorption began promptly and continued for several hours. About 1% of the oral dose appeared in the urine in 24 hours. (2) Radioactive magnesium was infused intravenously using a standardized semi-loading technic because of the low specific activity. Urinary excretion during 21 hours after infusion was 10–12% of the dose. The fecal excretion was negligible. (3) The renal clearance of $Mg^{28}$ was elevated to 17 ml./min. in the presence of high serum magnesium levels induced by the loading infusion and then fell to 2–3 ml./min. when the serum concentration returned to normal. (4) The initial volume of distribution of intravenously administered magnesium is approximately equal to the extracellular fluid space. The total body pool of magnesium exchanges slowly; 8 hours after an intravenous dose, $Mg^{28}$ exchanged with 100 mEq. of body magnesium. Erythrocyte magnesium is nonexchangeable up to 10 hours. (5) Plasma radioactivity decay curves obtained in normal subjects under standardized conditions of intravenous administration (100 mg. magnesium infused over 30 min.) are quite reproducible. They can be resolved graphically into 3 components with half-lives of 15, 40 and 350 minutes. The two faster components may represent abnormal rates of distribution induced by the loading conditions. The slow component, which appears to be unaffected by loading conditions, probably represents a fundamental parameter of magnesium metabolism—the rate of intracellular penetration. (6) Studies of magnesium kinetics in diabetes mellitus and myxedema reveal departures from the normal pattern.

### Magnesium-Protein Relationship and Status of Ultrafiltrable Magnesium in Normal and Abnormal Human Sera

By *Ananda S. Prasad, Edmund B. Flink, Horace H. Zinneman and Robert McCollister.* Department of Internal Medicine, University of Minnesota, and V. A. Hospital, Minneapolis.

Among major cations in human sera, magnesium has been least investigated. Magnesium-protein relationship and ultrafiltrable (UF) magnesium in normal and abnormal sera were studied.

Total and UF magnesium and protein fractionations (by paper electrophoresis) were determined. Ultrafiltration was done by a technic reported previously. In vitro binding in normals was studied by determining total and UF magnesium in protein fractions obtained from starch-block electrophoresis and incubated with magnesium solution. In vivo binding was studied in

three groups; Gr. I–normals, Gr. II–hypergammaglobulinemia exclusive of myeloma and Gr. III–hypoproteinemia. Total protein in Gm./Kg. $H_2O$ in Gr. I and III and albumin in mM/Kg. $H_2O$ in all groups were plotted against non-UF magnesium in mEq./Kg. $H_2O$ on arithmatic paper. Different globulins expressed as % of total protein were plotted against non-UF magnesium. Curves were drawn by method of least squares. Correlation coefficient (r) was calculated.

*In vitro albumin and alpha$_{I \& II}$-bound magnesium.* For total protein and non-UF magnesium $r = 0.75$ ($\pm 0.1$), suggesting that magnesium-protein relationship followed law of mass action and/pKMgProt in normals was 1.927 ($\pm 0.052$). From albumin-non-UF magnesium scattergram, roughly 0.01 mEq. of magnesium/Gm. of albumin was bound. Besides albumin in all groups, in Gr. I–alpha$_{I \& II}$, in Gr. II–alpha$_{II}$ and probably beta and in Gr. III–alpha$_{II}$ and beta appeared to bind magnesium.

In normals roughly 60–65% of non-UF magnesium is bound with albumin, 20–25% to globulins and the rest is independent of proteins.

In normals mean values for total and UF magnesium were 1.809 ($\pm 0.132$) and 1.175 ($\pm 0.096$), respectively. Ultrafiltrable magnesium was decreased in some cases of delirium tremens, nephrotic syndrome, liver cirrhosis, lupus erythematosus and hyperthyroidism. It was increased in uremia. In some patients with delirium tremens, diabetes, multiple myeloma and lupus erythematosus increased. UF magnesium was probably related to associated renal dysfunction.

### The Urinary and Fecal Excretion of Orally Administered $Mg^{28}$

By *Jerry K. Aikawa, Eloise L. Rhoades and Gerald S. Gordon.* Department of Medicine, University of Colorado School of Medicine, Denver. (Aided by a contract with the U. S. Atomic Energy Commission and a grant-in-aid from the American Heart Association.)

The results of previous studies of renal excretion of magnesium following its oral administration are conflicting. The availability of a new synthetic radioactive isotope of magnesium, $Mg^{28}$, with a half-life of 21.8 hours and high energy beta and gamma rays, has made possible a re-evaluation of this problem. The purpose of this study was to follow the behavior of orally administered magnesium, using $Mg^{28}$ as a tracer.

Ten to 25 $\mu$c. contained in 3 to 10 mEq. of magnesium were given orally to 26 subjects, 12 hospitalized adult males and 14 medical students, and serial specimens of blood, urine and feces were assayed for radioactivity content. The mean urinary excretion of radioactivity during the first 24 hours was 2.45% of the oral dose, that between 24 to 48 hours was 2.38%, and that between 48 to 72 hours was 0.71%. The maximal excretion during the initial 24 hours was 6.27%, and that for the entire 72-hour period of observation was 8.17%.

The maximal concentration of radioactivity in plasma occurred at 4 hours, but the actual increase in serum magnesium concentration was negligible, being 1 to 4 x $10^{-5}$ mEq./L. When complete fecal collections were extended up to 120 hours, as much as 88% of the administered radioactivity was recovered in the stool. Maximal recovery of 96% in urine and feces through 120 hours was obtained in the medical student who was the most cooperative and conscientious. The low renal excretion of magnesium is believed to be due to poor gastrointestinal absorption of this material. Previous investigators have reported excretion rates varying between 5 and 40%; the present study reveals a uniformly low urinary excretion rate.

### Norethandralone: Gonadotrophin Suppression Without Androgenic or Estrogenic Activity

By *Robert B. Leach, C. Alvin Paulsen, John Lanman, Norman W. Goldston and William O. Maddock.* City of Detroit Receiving Hospital, Detroit, and Department of Medicine, Wayne State University, College of Medicine. (Aided by a grant from the USPHS.)

Heretofore, significant gonadotrophin suppression has been observed only with potent androgens or estrogens. To determine possible gonadotrophin suppressing, androgenic and estrogenic effects of norethandralone, urinary gonadotrophin, estrogen and 17-ketosteroid excretion studies were performed along with clinical observations in 17 patients receiving this steroid. A daily oral dose of 20 to 100 mg. was administered for 2 weeks to 4 months to 5 castrated and 3 postmenopausal women, 1 functional uterine bleeder, 5 infertile but otherwise normal males and 3 eunuchoidal males. Urinary gonadotrophin excretion, which was normal or elevated before treatment, decreased to undetectable levels in all patients who received 50 to 100 mg. for 3 weeks or longer. Four of the 5 infertile men developed impotence during the first month of therapy.

Numbers of seminal fluid sperm which ranged from 10.5 to 59 million/cc., fell to less than 1 million/cc. in 3 patients, and to 4 million in one patient, by the 2nd month of therapy. Androgenic effects were not observed in any of the 3 eunuchoids, although each had been previously maintained on adequate doses of androgen. Apart from transient acne in one patient, none of the women developed androgenic effects. Estrogenic effects were not observed. None of the 8 men developed gynecomastia. In the 8 postmenopausal and castrate women, menopausal symptoms were not affected. Estrogen excretion, studied in 11 patients, was not significantly different from pretreatment levels. It is concluded that norethandralone, in amounts devoid of significant androgenic or estrogenic effects, is an effective suppressor of pituitary gonadotrophin secretion. It may have clinical usefulness in conditions where inhibition of pituitary gonadotrophin secretion is considered desirable.

### Protein Anabolism in Potassium Deficiency

By *Euclid G. Herndon, Jr., Milton E. Rubini and William H. Meroney.* Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C.

The dependence of nitrogen storage and normal growth on the simultaneous availability of potassium may be inferred from studies of starvation, catabolic stress, mammalian tissue analyses and growth of immature animals. The premise that nitrogen and potassium are lost or gained in relatively constant ratio was tested in adult man by study of nitrogen balance when dietary potassium was restricted.

Normal individuals given adequate protein (1.0 Gm./Kg.) could maintain nitrogen equilibrium on normal or reduced caloric intake when potassium was removed from the diet and over 400 mEq. of potassium were lost. To exaggerate anabolic stimuli during potassium deprivation two hypogonadal males were given testosterone. The dissociation of nitrogen retention from potassium storage was greatly exaggerated in the hypogonadal subjects. Each subject retained in excess of 4 Gm. of nitrogen/day and gained a calculated 2 Kg. of protoplasm. Despite continuing external loss of potassium gross muscular, prostatic and penile growth was noted. Although the androgen effect lessened slightly after the first week, repletion of potassium reaccelerated nitrogen storage. In one hypogonadal subject little phosphorus was retained with nitrogen until potassium was repleted. In the other hypogonadal subject who had severe osteoporosis, calcium and phosphorus were avidly retained during testosterone therapy. Plasma urea and potassium content was unchanged.

The data indicate that large amounts of nitrogen can be retained without potassium and militate against any broad interpretation of a constant potassium/nitrogen ratio.

### Isocaloric Substitution of Carbohydrate for Dietary Protein: Effects on Serum Lipids and Lipoproteins and the Response to Androgen Administration

By *Robert H. Furman, R. Palmer Howard and Leonard N. Norcia.* Cardiovascular and Endocrinology and Metabolism Sections, Oklahoma Medical Research Foundation, and Departments of Medicine and Biochemistry, University of Oklahoma Medical Center. (Aided by grants from the National Heart Institute and the Oklahoma State Heart Association.)

Epidemiologic surveys suggest an association between low serum cholesterol levels and a reduced mortality from atherosclerotic heart disease which is popularly attributed to a chronically reduced intake of fat. Because low fat diets are usually low in protein as well, this study was undertaken to determine the effect of protein deprivation on serum lipids and lipoproteins.

Since androgen administration depresses the ratio of high density/low density lipoproteins, a phenomenon of possible relevance to the proclivity of the male to coronary arteriosclerosis, the effects of methyltestosterone administration in the absence of dietary protein were also studied.

A "complete formula" containing corn oil (40% of calories), skim milk protein (14% of calories) and glucose was employed under metabolic balance study conditions. Serum lipoproteins were separated by differential ultracentrifugation and analyzed for cholesterol and phospholipid.

*Summary of results:* Feeding the "complete formula" resulted in reduction in serum cholesterol and phospholipid to values approximately 75% of those observed during the conventional diet. This reduction is attributable to the regulated intake of an unsaturated fat.

The isocaloric substitution of glucose for protein resulted in further reduction in serum lipids to values approximately 50% of those observed during the conventional diet.

The reduction in serum lipids noted when

dietary protein was withdrawn is attributable in most subjects to disproportionate reduction in beta lipoprotein lipid content.

In the absence of dietary protein, methyltestosterone administration: (a) leads to further reduction in serum lipids to levels 25–40% of those characterizing the conventional diet period; (b) does not cause the anticipated fall in alpha/beta lipoprotein ratios; (c) does not result in creatinuria.

*Conclusions:* Dietary protein is an important determinant of serum lipid levels. In the absence of dietary protein, methyltestosterone administration does not result in reduction in alpha/beta lipoprotein ratios or in creatinuria.

**Intravenous Triglyceride Tolerance Test as a Measure of Triglyceride Clearance from the Serum**

By *Philip C. Johnson and Carl W. Smith, Jr.* Radioisotope Service, V. A. Hospital, Oklahoma City.

The rate of clearance of fat, unlike glucose, from the serum is many times more rapid than is the rate of absorption of fat from the gastrointestinal tract. Because of this fact, oral fat tolerance tests are more a measure of fat absorption than of fat transport or clearance. To overcome this difficulty, we have attempted to obtain an expression of clearance of triglyceride from the serum by the rapid intravenous injection of 40 cc. of Lipomul containing 6.0 Gm. of triglycerides. Fasting, 10, and 20-minute serum samples are analyzed for triglycerides (Van Handel and Zilversmit). The percentage remaining is determined by dividing the triglyceride excess of the 20-minute sample by the excess of the 10-minute sample. In 13 normal patients, 72% ± 11 (2 S.D.) of the triglyceride excess remained 10 minutes later. In 3 patients with myxedema 88%, 83% and 81% remained, whereas after 5 days of triodothyroine, 0% remained. In one patient with myxedema under treatment with 160 mg. of desiccated thyroid daily, 0% remained. 94% remained in an active acromegalic without diabetes; in 3 patients with hypercholesterolemia, 60%, 41% and 24% remained. 98% remained in a patient with essential hyperlipemia.

This technic gives an expression that is probably related to the clearance of triglyceride from the serum and demonstrates that differences exist in various clinical states.

**The Relationship between Dietary Fat and Cholesterol Metabolism**

By *J. D. Wilson and M. D. Siperstein.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

The mechanism by which the ingestion of saturated fats elevates and unsaturated fats lowers cholesterol levels in both man and experimental animals remains unknown. Previous studies in our laboratory have demonstrated that this effect of variations in dietary fat cannot be due either to altered hepatic synthesis of cholesterol from acetate or to alterations in the biliary excretion of cholesterol or bile acids. The present investigation was undertaken in order to explore further pathways of cholesterol metabolism which might be influenced by saturated and unsaturated fats.

Groups of 4 rats were pair-fed isocaloric quantities of sterol-free diet containing no fat, 20% lard, or 20% corn oil. Four days after the beginning of the experiment cholesterol-4-$C^{14}$ was injected intravenously, and feces were collected from each rat for 14 days. The feces were dried and extracted for 24 hours with ethanol. Neutral sterols were then extracted into petroleum ether by the method of Rudman and Kendall; neutral $\beta$-sterols were precipitated from this fraction as digitonide. After saponification bile acids were extracted from the ethanol residue into ethyl ether. The $C^{14}$ content of each fraction was assayed in a liquid scintillation counter.

There was no consistent difference among the 3 groups in the cumulative excretion of either neutral $\beta$-sterols or bile acids. However, rats receiving corn oil excreted 3 to 8 times as much $\alpha$-sterols as rats receiving lard, and under these circumstances $\alpha$-sterol excretion may account for as much as 25% of the total $C^{14}$ excretion. Rats receiving no fat excreted only slightly more $\alpha$-sterols than the lard-fed group.

These studies demonstrate that under certain circumstances conversion of cholesterol to $\alpha$-sterols may be a major route of cholesterol breakdown. Furthermore, it is suggested that the cholesterol-lowering effect of unsaturated fats may result from this pronounced acceleration in $\alpha$-sterol excretion.

**The Effect of Pyridoxine Deficiency on the Incorporation of Radiocarbon into Liver Cholesterol**

By *Joseph M. Merrill.* V. A. Hospital, Nashville.

Interest in pyridoxine's place in lipid metabolism has been stimulated by the widespread use of unsaturated fatty acids to lower serum cholesterol. Rinehart's and Greenberg's produc-

tion of fibrous intimal plaques in monkeys fed diets deficient in pyridoxine and the revival of Virchow's idea that the first change in the production of atherosclerosis is alteration of connective tissue ground substance have created further interest in the role of pyridoxine in the pathogenesis of atherosclerosis.

To determine the effect of pyridoxine deficiency on the hepatic incorporation of radiocarbon into cholesterol, 3 groups of rats were fed the following diets: group I, a synthetic control diet containing 1.0 mg. pyridoxine hydrochloride per 100 Gm. of diet; group II, the same diet without pyridoxine; group III, the same diet as group I, but pair-fed with group II so that their growth curves were similar. After their weights remained unchanged for 4 weeks, the animals were injected with acetate-1-$C^{14}$. Thirty minutes later they were killed, the liver cholesterol digitonide recovered and its radioactivity (counts/sec./mg.) determined.

The average liver cholesterol digitonide radioactivity for the 3 groups of animals was as follows: group I, 2.134 (S.D. ± 0.8); group II, 6.306 (S.D. ± 2.47); group III, 0.937 (S.D. ± 0.05).

Group II rats had a 195% ($P < 0.001$) increase over group I in incorporation of radiocarbon into liver cholesterol. Group II rats had a 573% ($P < 0.001$) increase over group III in incorporation of radiocarbon into liver cholesterol. These results suggest increased hepatic synthesis of cholesterol in rats deficient in pyridoxine.

### The Effect of Acute Glucose Loads on Serum Cholesterol and Serum Lipid Phosphorus

By *E. Harvey Estes, Jr. and W. P. Wilson.* Departments of Medicine and Psychiatry, V. A. Hospital, and Duke University School of Medicine, Durham, North Carolina.

Glucose is known to play many important roles in the metabolism of lipids. The antiketogenic role of glucose is well known. Glucose is also known to facilitate the removal of particulate lipids from the blood, presumably by storage in adispose tissue. The present study reports the effect of glucose on serum cholesterol and phospholipid in human subjects.

Serum cholesterol and lipid phosphorus were studied during the hyperglycemic phase following oral and intravenous glucose loads in normal subjects.

Oral glucose (100 Gm.) produced an average drop of 15 mg. % in serum cholesterol ($p = <.001$) and of 0.69 mg. % in lipid phosphorus ($p = <.001$) in an average time of one hour (8 subjects).

Intravenous glucose (50 Gm. of 10% solution) produced an average drop of 25 mg. % in serum cholesterol, 1.30 mg. % in lipid phosphorus in 20 minutes (11 subjects). Both drops are highly significant ($p = <.001$). Intravenous normal saline in the same volume produced an average drop of 17 mg. % in serum cholesterol, 0.79 mg. % in lipid phosphorus (9 subjects), an effect which can be accounted for by hemodilution. Though the drop was greater with glucose than saline, the difference is not statistically significant ($0.1 > p > 0.05$). Oral glucose produces slight hemoconcentration; therefore, the above effects cannot be attributed to dilution.

The results indicate that oral glucose rapidly lowers serum cholesterol and serum lipid phosphorus to a significant degree. This degree of variability indicates that these lipid fractions are under more delicate regulatory control than previously suspected. The results also raise the possibility that low fat diets may lower cholesterol by virtue of their high carbohydrate content as well as their low fat content.

The results with intravenous solutions indicate that the state of hydration must be taken into consideration in evaluating the effect of various regimes on serum lipids.

### Metabolism of Chylomicron Phospholipids

By *Richard J. Havel and John C. Clarke.* Departments of Medicine and Surgery, University of California School of Medicine, San Francisco.

Chylomicrons administered intravenously to dogs are rapidly removed from the circulation. Chylomicron triglyceride and phospholipid disappearance rates are similar. Previously, it was found that during removal from the circulation of chylomicrons obtained from thoracic duct lymph of dogs fed palmitic acid-1-$C^{14}$, radioactivity accumulated in phospholipids of high density lipoproteins. Radioactivity was also found in high density lipoproteins after in vitro incubation of labeled chylomicrons with dog plasma. These observations suggested that labeled chylomicron phospholipids were exchanging with those of high density lipoproteins. Subsequently, in similar experiments using $I^{131}$-triolein or $P^{32}$, McCandless and Zilversmit (1957) reported that lymph phospholipid radioactivity disappeared from the circulation much more slowly than lymph triglyceride radioactivity. They also demonstrated in

vitro exchange of lymph and plasma phospholipids.

To evaluate the significance of these observations, we investigated the metabolism of chylomicrons doubly labeled with palmitic acid-1-$C^{14}$ (triglycerides) and $P^{32}$ (phospholipids). After intravenous injection, the half-times of disappearance were 5–10 minutes for $C^{14}$ and 2–4 hours for $P^{32}$. Transfer of chylomicron $P^{32}$ to unlabeled higher density plasma lipoproteins in vitro was rapid; half the $P^{32}$ was transfered by an exchange reaction in as little as 30 minutes. When large quantities of unlabeled chylomicrons were transfused into recipient dogs, phospholipids and triglycerides disappeared from the $S_f > 17$ lipoprotein fraction, which includes the chylomicrons, at the same rates, and there was no significant increase in $S_f$ 0–17 lipoprotein phospholipids. However, some accumulation of phospholipids was observed in high density lipoproteins during chylomicron removal.

These results are consistent with the interpretation that chylomicron triglycerides and phospholipids are metabolized by a common pathway. Exchange of chylomicron phospholipids with high density lipoprotein phospholipids largely accounts for the behavior of chylomicron $P^{32}$ in vivo. In addition, net accumulation of phospholipids in high density lipoproteins by an unknown mechanism occurs during chylomicron removal.

### Occurrence of Clearing Factor Inhibitor in Pregnancy and the Newborn

By *Chris J. D. Zarafonetis, Joseph Seifter, David Baeder and John Kalas.* Temple University School of Medicine, Philadelphia, and Wyeth Institute for Medical Research, Radnor, Pennsylvania.

Studies were undertaken to determine the possible role of lipid mobilizer hormone (LM) in connection with the known increase of plasma cholesterol during pregnancy. Plasma cholesterol and fatty acid determinations were made on blood from the mother (antecubital vein) during pregnancy, at term, and postpartum; on cord blood; and newborn vein (femoral) blood. LM was assessed by (1) bio-assay, and (2) in vitro determination of clearing factor inhibitory activity.

Occurrence of elevated plasma cholesterol and fatty acids during pregnancy was confirmed. Cord and newborn infant bloods held 1/3 or less the lipid values found in maternal blood at the time of delivery. Appropriate specimens taken during cesarean section yielded similar findings.

Increased plasma LM activity was found to be present during pregnancy. Of extreme interest was the finding that the LM content of cord and infant bloods was the same as that found in the respective maternal blood at the time of delivery. It appears, therefore, that LM traverses the placental barrier. These findings suggest that omental and possibly other lipid depots of infants were either depleted prior to birth or did not have an opportunity to accumulate during gestation. Limited necropsy experience indicates the omentum is thin and translucent at birth, which is consistent with either interpretation. It is also possible that placental and/or infant tissues have some other mechanism by which to prevent lipemia despite the presence of large amounts of lipid mobilizer hormone in the circulation.

One month or more post partum the plasma LM activity had declined to normal levels in all of the mothers tested.

### Plasma Clearing Factor Activity in Hyperlipemic States

By *William E. Connor and Mark L. Armstrong.* Department of Internal Medicine, State University of Iowa, and V. A. Hospital, Iowa City.

Clearing factor (CF) is a lipoprotein lipase thought to promote removal of dietary fat from the blood. A deficiency (Havel) or inhibition (Klein) of this enzyme has been postulated in idiopathic hyperlipemia. This study describes the activity of CF in 5 patients with idiopathic hyperlipemia, in 1 patient with nephrotic hyperlipemia, in 2 hypothyroid patients; and in rabbits with experimental hyperlipemia.

Plasma CF activity was produced by small doses of intravenous or subcutaneous heparin. Postheparin plasma, obtained periodically from 10 minutes to 24 hours, was incubated with coconut oil emulsion. After 2 hours of incubation, enzymatic activity was measured by glycerol production and by change in optical density of the plasma-coconut oil emulsion. Hyperlipemia in rabbits resulted from cholesterol-oil feeding.

CF activity invariably was found in 18 studies of postheparin plasma from patients with idiopathic hyperlipemia. Glycerol production up to 1.45 $\mu$M/ml. occurred in plasma after intravenous heparin. Twenty-four hours after subcutaneous heparin enzymatic activity was still demonstrable (0.36 $\mu$M/ml.). Comparable optical density changes occurred. These results were simi-

lar to 20 tests in 8 normal subjects. Hypothyroid patients likewise produced CF. Low activity in postheparin plasma of the nephrotic patient increased after addition of bovine albumin to the incubation emulsion. Hyperlipemic rabbits surpassed the CF production of normal rabbits. Plasma of patients with idiopathic hyperlipemia did not inhibit activity of normal plasma. After a single 50 mg. subcutaneous injection of heparin, CF persisted for 24 hours in many humans. It disappeared 4–6 hours after the same intravenous dose. CF activity from subcutaneous heparin remained long after the slight anticoagulant effect had disappeared.

These results suggest that the defect in hyperlipemic states is not a result of inability to produce CF under the conditions studied.

### The Specificity of Epinephrine-induced Lipolysis in Rat Adipose Tissue In Vitro

By *J. Earle White and Frank L. Engel.* Departments of Medicine and Physiology, Duke University Medical Center, Durham, North Carolina.

Epinephrine has been reported to increase plasma unesterified fatty acids (UFA) in man, presumably by promoting their release from adipose tissue. This interpretation has been supported by Gordon and Cherkes, and independently in this laboratory, by the demonstration that epinephrine promotes the release of UFA from rat adipose tissue in vitro. The present study assesses the physiologic significance of this effect by comparing the action of d-epinephrine, l-norepinephrine and d-norepinephrine with that of l-epinephrine for evidence of stereospecificity in tissue response.

Twenty to 30 mg. portions of intra-abdominal adipose tissue from fasted male rats were incubated in 1 ml. heparinized rat plasma at 36 C. in air in a Dubnoff Shaking Incubator. Plasma and tissue UFA were determined before and 3 hours after the addition of hormone or a distilled water control. Tissue from different animals varied in reactivity but results of repeated tests were proportionately similar. The liberated UFA, expressed in $\mu$M/100 mg. fat/3 hrs. were 0.19 $\pm$ 0.29 (S.D.) for the control in air. A representative assay revealed: l-epinephrine, 0.1 $\mu$g., 0.69, 1.0 $\mu$g., 1.44; d-epinephrine, 100 $\mu$g., 0.69, 1000 $\mu$g., 1.95; L-norepinephrine 0.1 $\mu$g., 0.73, 1.0 $\mu$g., 1.33; d-norepinephrine 50 $\mu$g., 0.65, 500 $\mu$g. 1.82. In a series of experiments l-epinephrine approximated l-norepinephrine in activity and each was from 100 to 1000 times as active as the respective d-isomer. A significant rise in UFA extractable from adipose tissue was induced by l-epinephrine, suggesting an action on lipolytic systems within the cell rather than facilitation of transport of UFA through cell membranes. Anaerobic incubation strongly inhibited epinephrine action but preincubation of tissue with dihydroergotamine failed to block the effect in air.

These data suggest a physiologic role of l-epinephrine and l-norepinephrine in mobilizing fat from adipose tissue.

### The Effect of a Pyrazinamide-induced Hyperuricemia on Serum Lipids

By *Alfred Kershbaum, Leonard J. Feinberg, Antonio C. deLeon and Samuel Bellet.* Division of Cardiology, Philadelphia Hospital, Philadelphia.

Serum uric acid levels are elevated to a significant degree in individuals with coronary artery disease and in individuals with hypercholesterolemia. The antituberculous drug, pyrazinamide, is known to cause a significant rise in serum uric acid. This study was undertaken to determine the effect of a pyrazinamide-induced hyperuricemia on serum lipids.

Ten subjects hospitalized with active tuberculosis were given pyrazinamide orally in a dosage of 2 Gm. daily for a period of 1 to 4 weeks. Blood serum was analyzed for uric acid, total cholesterol, phospholipids, and alpha and beta lipoproteins. The determinations were made before the drug was started, during the drug-induced hyperuricemia, and after the uric acid returned to pretreatment levels.

Hyperuricemia developed in all subjects given the drug, the uric acid rising to twice the control level in almost all instances. Two subjects with high serum uric acid before treatment showed similar elevations after pyrazinamide. The serum cholesterol, phospholipid, and lipoprotein concentrations did not significantly change during the period of hyperuricemia as compared to the lipid levels during the lower serum uric acid periods. In most instances the changes were less than 10% of the control values. In no instance did the serum cholesterol level rise with a rise in uric acid. The serum uric acid rose after one week of pyrazinamide administration and the hyperuricemia was maintained for periods of 3 days to 3 weeks. Benemid reduced the uric acid, but not to pretreatment levels. This did not affect the serum lipids.

Our findings indicate that high uric acid

blood levels induced by pyrazinamide do not significantly affect the serum lipid concentrations.

### The Biosynthesis of Oxalate from Glycine-1-$C^{14}$ in Man

By *James B. Wyngaarden and John V. Verner.* Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

Urinary oxalate excretion was measured in 11 patients with long-standing nephrolithiasis, and oxalate stones, by the method of Archer, Dormer, Scowen, and Watts. Four excreted normal quantities (12–45 mg./day, as calcium oxalate), 3 excreted between 50 and 100 mg./day, and 4 excreted more than 100 mg./day (128, 176, 230, and 238 mg./day, respectively). One additional patient excreted 700 to 1010 mg. of "apparent" oxalate per day, but gross pyuria was present. When the leukocytes were removed by filtration, the oxalate determination was 170 mg./day. By oxalate-$C^{14}$ isotope dilution analysis, including ether extraction of the initial precipitate, and recrystallization (Burns, Burch and King), his oxalate excretion measured 103–143 mg./day. These studies demonstrate one source of gross error potentially encountered with the Archer oxalate method; the nature of the substance in pus cells reducing permanganate has not been identified.

This patient was given 25 $\mu$c. of glycine-1-$C^{14}$ orally and urinary oxalate and urate were isolated. Oxalate was isolated from filtered urine following addition of carrier oxalate, and purified by ether extraction and recrystallization. Its $C^{14}$ content was maximal in the 0–12 hour urine specimen; after 48 hours urinary oxalate was devoid of isotopic enrichment. The labeled samples lost no activity on recrystallization, indicating the labeled substance was probably authentic oxalate. Peak calculated oxalate enrichment was 4440 c.p.m./m mole (as oxalic acid), compared with peak urate enrichment of 1300 c.p.m./m mole, present in the 60–72 hour sample. These results suggest that some of the urinary oxalate arose via the following pathway: glycine $\longrightarrow$ glyoxylic acid $\longrightarrow$ oxalic acid. A less direct route is, however, not excluded.

### Metabolism of Tritiated Thymidine in Man

By *J. R. Rubin, E. P. Cronkite, V. P. Bond, T. M. Fliedner and W. L. Hughes.* Medical Research Center, Brookhaven National Laboratory, Upton, New York. (Aided by the United States Atomic Energy Commission..)

Tritiated thymidine (T-Th) is incorporated into newly formed DNA of proliferating cells. This specific labeling technic is being used for autoradiographic studies of cell turnover and life-span in man for which the availability time of the label is essential. A selected terminal patient received 9 mc. of T-Th, 0.86 mc./mg., intravenously and the appearance and decline of nonvolatile tritium (NVT) and tritiated water (THO) activities in the plasma and urine were followed. Chromatography and counting were used to study the excretion of T-Th and other T- compounds. Plasma THO activity was maximal 40 minutes after injection and accounted for catabolism of 2 mc. of T-Th. A biologic half life of 9 days for THO was found as expected from the water balance, indicating that there was no significant increment of THO formed from tagged DNA or other retained T- compounds. T-Th appeared to be freely diffusable throughout plasma and red cells. Plasma NVT was maximal 1 minute after injection, declining to low levels by one hour. Urine NVT was maximal during the second hour, declined slowly for the next 2 hours and then declined more rapidly. Negligible amounts were present at 24 hours. Chromatograms of urine extracts were consistent with the presence of T-Th as well as other T- compounds not yet identified. The rapid disappearance of T-Th from plasma and the rapid evolution of THO suggest an availability time of T-Th for DNA synthesis of 1 hour or less. Autoradiographs of marrow smears showed good DNA labeling 19 minutes after injection. The absence of adverse clinical effects encourages further clinical investigation.

### Urinary Ribonuclease and Deoxyribonuclease: Variations during the Menstrual Cycle and Hormonal Influence

By *Joan M. Pappas and Anwar A. Hakim.* Department of Medical Research, National Children's Cardiac Hospital, Miami.

During studies on certain aspects of cellular metabolism we identified both ribonuclease (RNase) and deoxyribonuclease (DNase) activities in normal human urine. Since the menstrual cycle is characterized by alternating periods of rapid cell growth and involution of genital tissues, it was used in these studies.

Normal human urine was collected in 24-hour samples at intervals during the cycle and at defined periods during each of the 24 hours. The samples were frozen directly and lyophilized. Acid RNase, acid DNase, alkaline RNase and

neutral DNase were extracted and activities were determined on each sample. Thus the relative and total activity of each of the 4 enzymes were obtained during the 24-hour period and during the menstrual cycle.

The enzymic activity of crystalline RNase or crystalline DNase in presence of estradiol, estrone, progesterone, androsterone or testosterone was determined in parallel under two separate conditions: First, either crystalline RNase or crystalline DNase was incubated in vitro with each of the 6 hormones for 12 hours at 37 C. Second, either ribonucleic acid (RNA), or deoxyribonucleic acid (DNA) was incubated with each of the 6 hormones for 12 hours at 37 C. Ribonuclease or Deoxyribonuclease activity was then followed.

During the menstrual cycle the RNase activity showed a certain relationship to the excretion of estrogens into the urine. Two peaks of total RNase excretion, one prior to ovulation (11th day) and one associated with maturity of the corpus luteum (21st day) were obtained. The ribonucleases excreted during the first peak differed in specificity, optimum pH, action on RNA and heat stability. During the 1st to the 6th day of the menstrual cycle, both the acid and the alkaline DNase showed greater enzymic activity per day than during the 19th to the 28th day.

In vitro experiments indicated that diethylstilbesterol activated Rnase, while progesterone, estradiol, estrone, androsterone and testosterone inhibited RNase activity. All 6 of these compounds activated DNase.

The presence of RNase or DNase activity in the urine of normal persons, with a certain relationship to the excretion of sexual hormones, is of relevance in the clinical evaluation of certain physiologic disorders of abnormal cellular metabolism.

### Observations on the Metabolism of Radioactive Histamine in Man

By *Gildon N. Beall and Paul P. VanArsdel, Jr.* Department of Medicine, University of Washington School of Medicine, Seattle.

Ten to 100 $\mu$g. of $C^{14}$-labeled histamine were injected intravenously into hospitalized patients and normal medical students to investigate the metabolism of histamine in man. Urine and plasma samples were collected, prepared in a polyether solvent, and assayed in a Packard Tri-Carb Liquid Scintillation Spectrometer with an efficiency of 40 to 50%.

Most normal individuals excreted over 60% of the administered radioactivity into the urine during the first 8 hours after the injection. Subjects without kidney disease excreted all of the radioactivity in 48 hours. One patient with uremia, due to chronic nephritis, excreted less than 20% of the carbon$^{14}$ in 48 hours, and 12 days after injection was still excreting small amounts of radioactivity.

Plasma levels of radioactivity were similar in all the subjects. The average level during the first 30 minutes after the injection was 0.1 $\mu$g./100 ml. plasma when calculated as histamine base. However, less than 10% of this plasma radioactivity was chemically identical with histamine. No measurable plasma radioactivity followed the intravenous injection of 10 $\mu$g. of radioactive histamine. Concurrent administration of 3-beta-aminoethyl-pyrazole (Histalog) under these circumstances produced a significant, brief increase in plasma radioactivity soon after the injection. The rate of urinary excretion of the isotope in the presence of this analogue of histamine was significantly delayed.

The rapid metabolism of histamine is not influenced by the presence of allergic disorders in the patients studied to date. The delayed excretion in the patient with uremia is probably due to impaired renal clearance and not to lack of histaminase (diamine oxidase). Preliminary work with two-dimensional paper chromatography reveals two major metabolites in normal urine with migration similar to imidazoleacetic acid-riboside and 1-methylimidazole-4-acetic acid.

### Diurnal Variations in the Excretion of Urinary 5-Hydroxyindoles

By *Henry A. Johnsen, Jr., Ralph Eugene Smith and Werner Simon.* Departments of Medicine and Psychiatry, University of Minnesota, and the V. A. Hospital, Minneapolis.

Diurnal variations in blood and urine levels of several endogenous metabolites occur normally and probably involve a neurologic-endocrinologic relationship. Serotonin (5-hydroxytryptamine) has been implicated as an important neurohumor and is present in highest concentrations in the hypothalamus and brain stem of animals. Its concentration in the brain tissue of mice varies diurnally.

Diurnal variations in the metabolism of sero-

tonin in the human were studied by measuring the excretion of urinary 5-hydroxyindoles. Urines were collected every 3 hours for 3 days from 15 healthy, active men under the age of 40. Renal function was estimated as being grossly normal by means of urinalyses and BUN determinations. A regular diet was administered since fluctuations in dietary tryptophan do not significantly affect the production of 5-hydroxyindoles in normal individuals. Urines were personally collected by us throughout the nights and days for the entire 72-hour period, in order to insure accuracy. One patient with argentaffinomatosis was similarly studied.

A definite diurnal rhythm in the excretion of urinary 5-hydroxyindoles was observed in the normal subjects, which was repeated on the 2nd and 3rd days of collection. The interval of lowest excretion occurred between midnight and 3 a.m. A sharp rise in the amount of 5-hydroxyindole excreted occurred between 3 a.m. and 6 a.m. and was thus unrelated to activity or eating. The peak of 5-hydroxyindole excretion occurred in the 3-hour period on either side of noon. A diurnal variation was also noted in the patient with malignant carcinoid, but the rhythm was reversed, the peak of excretion occurring near midnight.

The results suggest that serotonin metabolism varies diurnally in the human, as in mice. A relationship to diurnal variations in other metabolites is suggested. When argent affiinomatosis is suspected, 24-hour urine collections are of greater diagnostic value.

**Acute Intermittent Porphyria: A Genetic Study (Preliminary Report)**

By *Alex T. Murphey, Paul E. Fitzpatrick and William S. Harms*, Department of Medicine, Medical College of Georgia, Augusta.

The suggestive family history obtained from a patient with acute intermittent porphyria led to an investigation of the familial aspects of this disease.

A geographically circumscribed and highly inbred family of about 500 people was discovered in south Georgia. A detailed family tree and history were constructed. Urines were analyzed qualitatively for porphobilinogen, uroporphyrin and coproporphyrin. The method of Watson and Schwartz was used for porphobilinogen. Porphyrins were determined by appropriate extraction and fluorescence with Wood's light.

Of 108 persons tested thus far, 16 were definitely positive for urinary porphobilinogen. Of those negative or doubtful for porphobilinogen, 17 demonstrated porphyrins in the urine. If these latter are considered to represent conversion of porphobilinogen to porphyrins, an incidence of 30.6% in this series is obtained. No sex linkage was noted.

The group is unusual in several respects: (1) a remarkable predominance of males affected by or dying of symptoms characteristic of acute intermittent porphyria; (2) an unusual number of children dying of symptoms strongly suggestive of the disease; (3) a distinctly unusual incidence of muscular dystrophy; and (4) a remarkable degree of consanguinity.

The trait appears to be transmitted as a Mendelian intermediate characteristic with different manifestations in homo- and heterozygous individuals.

## GASTROINTESTINAL SYSTEM

**The Determination of Gastrointestinal Calcium Exchange in Man on a Low Calcium Intake**

By *Herbert Moskovitz and Malcolm Stanley*. Departments of Medicine, New England Center Hospital, Tufts University School of Medicine, Boston, and University of Louisville School of Medicine, Louisville, Kentucky. (Aided by a grant from the National Institutes of Health.)

Gastrointestinal calcium exchange was studied 7 times in 6 normal paid subjects while taking a uniform liquid semisynthetic diet containing no phytate or added vitamin D, 2530 calories, 118 Gm. protein (egg albumin), 101 Gm. fat (olive oil), 250 Gm. carbohydrate (lactose), 53–103 mg. calcium and 160 mg. phosphorus daily. Addition to the diet of the inert indicator, $Cr_2O_3$, and of $Ca^{45}$ facilitated determination of fecal excretion and hence of intestinal absorption.

*Excretions* (fecal) of unabsorbed $Ca^{45}$ were 5–15% in 5; in 2 others, 44% and 25% were excreted. Concomitantly 56–190 mg./day of stable calcium were excreted. *Absorptions* of stable calcium were 612–1586 mg./day in those whose

absorptions of $Ca^{45}$ were 85–95%; the others absorbed 218 (56%) and 496 (75%) mg./day. *Secretions* were 715–1672 mg./day in the first group and 387 and 658 mg./day respectively, in the other 2.

Calculations of absorption and secretion were based upon assumptions that dietary stable and radioactive calciums were homogenously mixed with the calcium secreted from the salivary glands, stomach, pancreas, bile and upper intestine, that absorption did not occur before complete mixing of all components, that absorptions of $Ca^{45}$ and stable calcium from various components were similar, and that significant further secretion of stable calcium did not occur distal to the site of beginning absorption. Resecretion of absorbed $Ca^{45}$ was readily corrected for.

As it is unlikely that all of the above assumptions are true, values given are maximal for absorption and secretion of stable calcium. However, it is emphasized that absence of phytate, very low calcium and phosphorus, and large lactose content of the diet favored maximal calcium absorption from the gut. This was substantiated by the large proportion of $Ca^{45}$ absorbed in the majority of subjects.

### Paper-Electrophoretic Analysis of the Secretion from the Explanted Gastric Mucosa in Rats

By *George B. Jerzy Glass and Stanley C. Skoryna.* Department of Medicine and Gastroenterology Research Laboratory, New York Medical College, Flower and Fifth Avenue Hospitals, New York, and Department of Experimental Surgery, McGill University, Montreal.

Webster, Toovey and Skoryna developed a method for the exteriorization of the flaps of the rat stomach. Histologic examination showed that in about 8 weeks the overgrowing surface epithelium covered the orifices of the gastric glands. Thus, a unique experimental condition was produced for collection of the secretion of gastric surface epithelium.

We applied this technic to the study of the derivation of high molecular components of gastric juice by explanting 12 rats and collecting their individual secretions. As controls, we used a large group of normal rats, in whom, after a starvation period of 24 hours, we ligated the cardiac and pyloric ends of the stomach. The animals were sacrificed 24 hours later and gastric juices were recovered. After determination of HCl and pepsin, each collection was dialyzed, lyophilized and submitted to paper-electrophoresis by the method of Glass et al. in Spinco cells, against borate buffer (pH 9.0, ionic strength 0.12) on Whatman #1 paper, at 0.4 mA/cm., 120 v., for 5½ hours. The strips were stained with amido black 10B, SF light green and PAS stains, and traced and integrated in Analytrol.

The secretion of the explants contained neither free HCl nor pepsin, in line with previous observations of Webster et al. On electrophoresis, it contained neither the fast anodic protein component of mobility of pepsin nor the fast anodic carbohydrate material, both present in gastric juice of normal rats. This indicates the glandular origin of these two components.

The gastric juice of normal rats, on the contrary, as well as the secretions of the explants, contained the mucoproteose fraction of gastric mucin and, on electrophoresis, in high concentration, the slow anodic protein and carbohydrate materials and the cathodic component of mobility of peaks $M_3$ and $M_4$ of human gastric juice. This suggests the derivation of these materials from the surface epithelium. Another anodic component of intermediate anodic mobility was present in the secretion of gastric explants. It resembled the surface epithelial material $M_2$ of human gastric juice in its location. However, it had the mobility and staining properties of serum albumin, and probably represents serum albumin which has transudated through the explanted thin layer of surface epithelium, as it does through the thinned mucosa of some patients with gastric atrophy.

### The Protein Distribution of Gastric Juice after Electrophoresis: A Distinctive Pattern in Pernicious Anemia and Achlorhydria

By *Irwin Katzka.* Medical Department, Long Island Jewish Hospital, New Hyde Park, New York. (Aided by a grant from the Rosenstock Foundation.)

This report describes studies on the quantitation and characterization of the large molecular components of human gastric juice. Fresh gastric juice from 10 healthy subjects and 16 patients with disease states such as peptic ulcer, pernicious anemia or functional achlorhydria was subjected to electrophoresis using the starch zone method. A microassay for protein permitted the use of 6 to 8 ml. of unconcentrated gastric juice.

The protein patterns for normal gastric juice showed 4 major concentration peaks (A, B, C and D.) The latter two moved toward the anode; peak B showed central mobility and peak A was

cathodic. The electrophoregrams from patients with peptic ulcer were similar to normals except that the C peak was generally higher. The electrophoregrams of patients with pernicious anemia and functional achlorhydria showed characteristic differences. The A, C and D peaks were minimal or absent, while the B peak was unusually large. Saliva electrophoregrams showed a pattern similar to that of gastric juice in achlorhydric patients. It is proposed that the A, C, or D peaks contain a proteolytic enzyme which digests the B peak in normals. When this enzyme(s) is absent as in pernicious anemia, the B peak from the saliva and/or gastric mucosa, persists. This pattern is found in all histamine achlorhydrics with or without pernicious anemia.

**The Effect of Amphenone upon Gastric Activity**

By *Julian Arabehety, Jorge Manrique, Paul Parades and Seymour J. Gray.* Department of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School, Boston.

Diminished gastric activity has been observed in humans and in animals after bilateral adrenalectomy. Since amphenone B (3, 3-di (p-aminophenyl) butanone-2 dihydrochloride) has been shown to inhibit adrenocortical function in animals and in humans, the present study was undertaken to determine its effect upon gastric activity.

A single subcutaneous injection of amphenone to pyloric ligated rats produced a significant decrease in the volume of gastric secretion, free gastric acid concentration, potassium content and an increase in pH, gastric juice sodium and pepsin concentration. The injection of cortisone, corticosterone, DOCA or ACTH with amphenone did not alter the amphenone effect. Aldosterone did not alter gastric secretion except for a reduction in gastric juice sodium. When amphenone was injected with aldosterone, however, the usual amphenone effects upon gastric juice were again observed. When amphenone was administered with histamine, the amphenone effects relating to pH and free acid concentration were not observed.

Adrenalectomy produced a decrease in gastric volume, acidity and pepsin secretion, and an increase in gastric juice sodium and pH. The inhibition of gastric activity by amphenone was similar to that seen after 10 days of adrenalectomy. In previously adrenalectomized rats the amphenone inhibitory effects upon gastric secretion were absent.

An increase in adrenal gland weight was observed 4 hours after amphenone administration, but was not observed when cortisone, corticosterone, DOCA, aldosterone or histamine were administered with amphenone. ACTH alone or with amphenone produced an increase in adrenal weight.

Amphenone produced a considerable increase in thymus weight which could be prevented by the simultaneous injection of corticosterone, aldosterone or histamine, but not by DOCA or adrenalectomy. ACTH or cortisone alone or in combination with amphenone decreased thymus weight. Previous adrenalectomy with or without amphenone resulted in a considerable increase in thymus weight.

**Electrogastrographic and Radiologic Studies in Patients with the Dumping Syndrome**

By *Edmund N. Goodman, Ralph Schlaeger, Harold D. Harvey and Henry Colcher.* Gastrointestinal Research Laboratory, Departments of Medicine, Radiology and Surgery, College of Physicians and Surgeons, Columbia University, and Medical, Radiological and Surgical Services of Presbyterian Hospital, New York. (Aided by grants from N.I.H., Rothschild Research Gift, James O. McCue Research Gift and St. John Special Fund.)

Asymptomatic, gastrectomized patients and those exhibiting manifestations clinically classified as the "dumping syndrome" were investigated radiologically and by the electrogastrographic method. The group consisted of 52 patients having undergone partial gastrectomy with various forms of restoration of continuity. Radiologic evaluation was concerned with the physiology of the gastric remnant, including its function as a reservoir, and mechanisms of emptying. In the fasting state, fluoroscopic examination was performed utilizing a nonflocculating barium suspension; the procedure was repeated immediately following the ingestion of a ham sandwich.

No significant difference in the range of variation in gastric pouch emptying was observed in symptomatic as compared to asymptomatic patients or in the various forms of restoration following resection. In the gastric remnant, no peristaltic waves were observed, although emptying invariably was immediate when a liquid medium was employed. With a standardized solid meal, the pouch demonstrated striking reservoir capacity for the solids, while additional aliquots of a barium suspension passed immediately

through the stoma. The gastric remnant remained aperistaltic as well, when serving as a distended reservoir for solids. Propulsive waves were observed only in the small intestine adjacent to the anastomotic site.

Electrogastrographic recordings were obtained in this group of patients by use of a double lumen tube equipped with 6 electrodes mounted on a rubber balloon. Intraluminal volume and pressure changes were recorded simultaneously with the electrical activity of 6 areas of the stomach or small intestine.

Electrical activity with frequent runs of classical 3/min. waves and variations in intraluminal pressure suggestive of gastric peristalsis were seen in the asymptomatic group. Seven patients, presenting symptoms classified as "dumping," exhibited large irregular electrical waves distinctly different from the normal pattern.

### Further Observations on the Pathogenesis of Hypercalcemia Secondary to Calcium Carbonate ($CaCO_3$) Ingestion in Duodenal Ulcer Patients

By *Charles R. Kleeman, Robert E. Rockney, Morton H. Maxwell and Morton I. Grossman.* Department of Medicine, V. A. Center, and University of California Medical Center, Los Angeles.

It is now clear that $CaCO_3$, as the sole antacid ingested, can cause hypercalcemia in some duodenal ulcer patients (Wenger et al., 1957; Kleeman et al., 1958). Hypercalciuria may or may not occur and renal impairment and/or alkalosis are not essential prerequisites.

To clarify this disorder further $CaCO_3$ (20–40 Gm. daily) has now been administered to 6 duodenal ulcer patients (3 "hypersecretors"), 8 normals, and 6 patients with moderate chronic renal disease for 5–7 days. Results: (1) hypercalcemia did not develop in any of the subjects although hypercalciuria occurred in all (300–800 mg./24 hrs). This level of hypercalciuria was equal to or *greater* than that attained by the hypercalcemic subjects previously reported. (2) Moderate renal impairment *did not* predispose to hypercalcemia, although hypercalciuria was less than in the normals. (3) When parathyroid extract (800–1000 units/day) was administered to the normal subjects simultaneously with the $CaCO_3$, hypercalcemia (11.5–15.0 mg. %) always developed, but the magnitude of the hypercalciuria was *no greater* and at times *less* than that observed in the normocalcemic control not receiving parathyroid extract. (4) Gastric hypersecretion did not predispose to hypercalcemia.

A review of 24 reported cases of "Burnett's Syndrome" (hypercalcemia secondary to excessive milk and alkali ingestion) disclosed: (1) Histologic hyperplasia of the parathyroids was present in 6 of the 7 examined cases. (2) Moderate hypercalciuria was present in 5 of the 8 cases in which it was measured. (3) *Prior* renal disease was conclusive in only 5 cases.

This investigation suggests that those subjects with duodenal ulcer who develop hypercalcemia after $CaCO_3$ ingestion and possibly those with "Burnett's Syndrome" are unable to normally or adequately inhibit the release of parathyroid hormone when challenged with large chronic loads of calcium. In these subjects the magnitude of the hypercalciuria is less than expected for the level of the hypercalcemia because homeostatic inhibition of parathyroid hormone release is essential for *increasing* the renal *clearance* of calcium.

### The Association of Peptic Ulcer and Hereditary Hyperparathyroidism

By *Charles E. Jackson.* Caylor-Nickel Clinic, Bluffton, Indiana.

Following an experience with 6 and possibly 7 cases of hyperparathyroidism in 2 generations of one family (associated in at least 2 instances with recurrent pancreatitis), hyperparathyroidism was found to be present in a 31-year-old man with a ureteral calculus and his 55-year-old father with recurring peptic ulcer. Peptic ulcer was also present in others of this family, with hyperparathyroidism being excluded in all the ulcer cases except a paternal uncle and his daughter who had expired before this study was undertaken. The occurrence of peptic ulcer in this pedigree in individuals with hyperparathyroidism and in others without hyperparathyroidism provides additional suggestive evidence that peptic ulcer may be an inherited condition. It is possible that some factor present in the hyperparathyroid individual causes the individual's underlying inherited ulcer tendency to be manifest more completely. If the ulcer tendency were completely or almost completely manifest in hyperparathyroidism, then the high incidence of the association of ulcer and hyperparathyroidism might be a reflection of the frequency of the peptic ulcer tendency in the general population.

### Histopathology of the Duodenum

By *R. D. Schwartz, R. Yesner and H. M. Spiro.* Yale University School of Medicine, New Haven.

The Shiner suction biopsy tube makes possible the study of human small bowel mucosa without the autolysis characteristic of autopsy material. This study is an attempt to characterize the histopathology of the duodenum in a spectrum. Significant changes, from the normally tall, slender villi to those clubbed and atrophied in advanced sprue, are in degree, not in kind, of pathology.

Eighteen patients have thus far been studied. In achylia gastrica, pernicious anemia, regional enteritis, and ulcerative colitis, the duodenal mucosa showed normally delicate slender villi somewhat shorter than those in the jejunum. Of 5 patients with chronic longstanding diarrhea, of unidentifiable cause, 1 had normal duodenal histology, 1 had mild mucosal hypoplasia and edema, and 3 showed marked variation in the villous pattern. One patient with chronic relapsing pancreatitis demonstrated thickening, edema, and confluence of the villi.

In sprue, the duodenal mucosa showed broad flattened villi in 1 patient, and in another almost complete atrophy of the villi. Such a pattern is histologically undifferentiatable from that seen in other chronic diarrheas, although such changes are apparently more marked in longstanding severe sprue. Only in 1 patient with Whipple's disease were absolutely specific changes observed: enormously enlarged villi stuffed with PAS positive macrophages occurred in no other biopsy material.

It is believed, therefore, that the histopathologic changes in sprue though suggestively characteristic are nonspecific and represent only an advanced stage of a pathologic process apparently common in the duodenum.

### Studies of Gut Mucosa: The Effect of Endotoxin on Rat Intestine

By *S. Broitman, A. Bezman and N. Zamcheck.* Gastro-intestinal Research Laboratory, Mallory Institute of Pathology; Gastro-intestinal Clinic, Boston City Hospital; and Department of Pathology, Boston University School of Medicine.

The effect of endotoxin on the "mucosal barrier" of the rat small intestine was studied.

Twenty to 80 minutes following the intracardiac injection of 6.5 $MLD_{50}$ of Salmonella-typhi endotoxin into 200-Gm. rats, edema and marked congestion of the intestine and engorgement of the mesenteric vessels were observed. These were accompanied by microscopic mucosal hemorrhages and benzidine positive stools. Seventy-five % decrease in oxidation of small intestinal mucosal homogenate in the presence of succinate and rat serum (the latter necessary to obtain consistent succinoxidase values) occurred within 30 sec. after injection. Activity remained at this low level until the animal was moribund (80 to 120 min. later). Endotoxin in vitro did not inhibit the succinoxidase activity.

In order to relate these findings to intestinal permeability to bacteria a culture of Serratia marcescens (a pigmented, nonpathogenic, non-indigenous, small gram negative rod) containing $3 \times 10^8$ organisms was innoculated into the rat stomachs 45 min. after intracardiac injection of endotoxin. When the animals were killed, 15 min. later, kidney, liver, spleen and heart were sterile on culture, whereas the organisms were always recovered from the small bowel lumen. The presence of effective extramucosal antibacterial mechanism(s) was excluded by the intracardiac injection of minimal numbers of Serratia marcescens (150 per cc. of circulating blood) and the finding of positive cultures of these bacteria in the visceral organs.

The resistance of the mucous membrane to the passage of micro-organisms despite the morphologic and enzymatic alterations produced by rapidly lethal doses of endotoxin is one indication of the effectiveness of this biologic barrier.

### Small Intestinal Motility Recorded by Means of a Radiotelemetering Capsule

By *James S. Bernstein and John T. Farrar.* Medical Service, V. A. Hospital, New York, and Department of Medicine, Cornell University Medical College.

Methods which have been used to detect and record intraluminal pressures within the gastrointestinal tract in normal human subjects require the passage of long tubes through the mouth, nose or anus. These methods possess inherent defects which have prevented thorough study of small intestinal and colonic motility.

An ingestible, pressure-sensitive radiotelemetering capsule has been developed which permits permanent recording of intraluminal pressures of the stomach, small intestine and colon without connecting wires or tubes. The capsule is a rigid

plastic cylinder, 3.0 cm. long and 1.0 cm. in diameter, and contains a replaceable battery, a frequency-modulation transistor radio transmitter and a pressure-sensitive diaphragm which responds to frequencies up to 10 c.p.s. Intraluminal pressure on the diaphragm is transmitted by means of radio signals to a frequency-modulation receiver. The pressures are displayed constantly on an oscilloscope and recorded permanently on paper.

The capsule has been swallowed without difficulty 26 times by 18 patients and passed through the gastrointestinal tract without causing any subjective sensation. Gastric and right colonic pressures have been recorded in many of these, but the emphasis has been on the small intestinal records. In a series of 13 subjects, including normals, patients with functional gastrointestinal disorders and patients after gastrectomy, intraluminal small intestinal pressures have been recorded for periods ranging from 1 to 5 hours, usually more than 3 hours. During the study the subject sits or lies in any comfortable position, apparently free from disturbing influences. Examination of the small intestinal records reveals similarities to those recorded by other methods, but significant differences are also noted.

In addition to providing data on gastrointestinal motility, this instrument demonstrates a technic that may be applicable to the acquisition of other important chemical and physical data from within the gut.

### Islet Cell Tumor and a Syndrome of Refractory Watery Diarrhea and Hypokalemia

By *John V. Verner and Ashton B. Morrison.* Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

The object of this report is to draw attention to a syndrome of watery diarrhea, hypokalemia, and death from renal failure in association with islet cell tumor of the pancreas. Zollinger and Ellison have described a syndrome of refractory peptic ulceration in patients with islet cell tumors, but the early occurrence of diarrhea in some of the cases reported has not been clearly recognized and discussed. Two cases are reported which manifested diarrhea, hypokalemia, and death from vacuolar nephropathy in which no peptic ulceration was found.

*Case I.* A 67-year-old Negro male had a 10-month history of diarrhea and extreme weight loss. Gastrointestinal x-rays were negative and blood chemical findings showed hypokalemia and azotemia. At autospy a 4x4 cm. tumor of the pancreatic islets composed of alpha-like cells was found. There was a small pituitary chromophobe adenoma and the renal tubules showed vacuolar changes. There was no peptic ulceration.

*Case II.* A 19-year-old white male had a 3-year history of explosive watery diarrhea and hypokalemia requiring 16 hospitalizations. The diarrhea was only transiently improved after hydration and potassium administration. A final exacerbation of diarrhea led to death from uremia. At autopsy a 2x2 cm. islet cell tumor was found which contained no beta cells. Vacuolar changes were seen in the renal tubules and there was no peptic ulceration.

Seven additional cases of islet cell tumors in whom diarrhea was an early and prominent symptom have been collected from the literature. In only 3 cases were peptic ulcers found in addition to the diarrhea.

Patients with refractory watery diarrhea and hypokalemia may have underlying islet cell tumors.

### Jejunal Biopsies in Primary Malabsorption Syndrome and Related States

By *Chuni Wang, Hillard W. Himes and David Adlersberg.* Department of Medicine, Mount Sinai Hospital, New York.

Jejunal biopsy by the oral route (Shiner tube) was performed in 20 persons, 10 men and 10 women, whose ages ranged from 14 to 73 years. Of these, 10 were patients with idiopathic malabsorption syndrome (primary sprue), whose diagnosis was established by the standard clinical and laboratory criteria. The majority of them have been under our observation for many years. Two patients had diarrhea of unknown etiology and one each had scleroderma, pernicious anemia, pancreatogenous steatorrhea (caused by gastric carcinoma involving the pancreas), unusual form of jejunoileitis, inactive celiac disease and diarrhea after total gastrectomy. Two persons were "healthy" individuals.

Striking changes were encountered in patients with primary malabsorption syndrome. They consisted of marked atrophy of the mucosa. The villi lost their characteristic finger-like configuration, and were clubbed and flattened. The surface epithelium covering the villi changed from the usual columnar type to a cuboidal shape. The nuclei were irregular in shape and position in the cells. There was an increase in the number of the goblet cells. In the lamina propria, there

was increased cellularity, consisting mainly of lymphocytes, plasma cells and sometimes eosinophils. In some patients these changes were patchy in distribution. It is of interest that the patient with scleroderma showed milder abnormalities in the villi whereas other patients with inactive celiac disease (14 years old), pernicious anemia and pancreatogenous steatorrhea presented essentially normal appearance of the jejunal mucosa.

In addition to the regular hematoxylin eosin stain various staining technics for acid mucopolysaccharides and nucleic acids were employed.

### The Use of a Standardized Carotene Loading Test in the Diagnosis of Malabsorptive States

By *Paul R. Finley, Richard Doe and Ruth Doyle.* Departments of Medicine and Pathology, V. A. Hospital, and University of Minnesota Medical School, Minneapolis.

A simple but reliable screening test for the detection of malabsorptive states involves the administration of carotene in a standardized challenge dose. Because carotene absorption is closely parallel to the absorption of fat from the gastrointestinal tract, the observed rise of carotene in the plasma should reflect the extent of malabsorption that may exist.

Carotene capsules (5,000 units) were given orally, 15,000 units t.i.d. for 3 days. Two fasting blood specimens were obtained, the first before carotene administration, the second, 4 days later. Plasma was extracted with petroleum ether and read directly in a colorimeter. Subjects included 35 men in whom no malabsorption was apparent, and 35 others who were afflicted with various malabsorptive states, including idiopathic sprue, chronic pancreatitis, cirrhosis, regional enteritis and ulcerative colitis. Ability to absorb fat was also tested by regulating the dietary fat and determining stool fat and nitrogen.

In 35 normal subjects, initial carotene levels ranged from 48γ% to 195γ%. Response to the challenge dose of carotene was manifested by elevation of plasma carotene, measured on the 4th day, of not less than 45γ% nor more than 117γ%. In many cases, the rise doubled the initial level. In 35 subjects with various malabsorptive states, initial carotene levels ranged from 0γ% to 123γ%, but the response to loading dose was never more than 29γ% and many times was less than 5γ%. Thus, overlap of normal and abnormal states was observed frequently when only fasting levels were considered, but absolutely no overlap was observed when the increment in plasma concentration before and after standardized loading was utilized.

The utility, simplicity and sensitivity of a standardized carotene loading test in the detection of the malabsorptive states make it a valuable adjunct in their diagnosis.

### Plasma Optical Density and Radioiodine Levels Following Oral Administration of $I^{131}$-Labeled Triolein

By *A. William Horsley, James A. Clifton, Titus C. Evans and William E. Connor.* Department of Medicine and Radiation Research Laboratories, College of Medicine, State University of Iowa, Iowa City.

Oral administration of $I^{131}$-labeled triolein (Raolein) with analysis of stools and blood for radioactivity has been shown to be a valuable method for detection of malabsorption syndromes. Osmon has reported differences in serum optical density (OD) between healthy subjects and patients with pancreatic insufficiency following a fat meal. Plasma OD is a measure of turbidity and represents the neutral fat fraction.

We have measured OD and radioactivity in the plasma of 12 healthy subjects and in 7 patients with a variety of malabsorption syndromes. A homogenized meal containing 50 ml. of corn oil, 240 ml. skim milk, and 50 μc. of Raolein was given following a 12-hour fast. Plasma was obtained every 2 hours for ten hours and the OD determined in a spectrophotometer. Radioactivity was measured in a well-type scintillation counter and expressed as % of the dose at each time interval.

Though plasma lactescence disappears rapidly after reaching a maximum in the normal group, the coefficients of correlation of the two methods were significant at the second hour ($r = .62$, $P = .05$) and fourth hour ($r = .80$, $P = .01$). A similar analysis for the abnormal group was not practical as half of the plasma values were zero. The mean of the maximal plasma radioactivity was $8.5\% \pm 1.9$ for controls and $1.3\% \pm 0.55$ for the abnormal group. The mean of the maximal change in OD was $0.261 \pm .127$ for controls and $0.057 \pm 0.47$ for the abnormal group. The difference between the means in each case is significant ($P = .001$).

The determination of plasma OD following a fat meal appears to be a reliable index of fat absorption in both healthy subjects and in patients with malabsorption syndromes.

### A Quantitative Comparison of Indices of Malabsorption: $I^{131}$ Triolein, Fat and Nitrogen Balance, Glucose and Vitamin A Absorption Curves

By *Arthur B. French, Makoto Ishikawa, Hugh S. Wiggins and H. Marvin Pollard.* Gastrointestinal Section, and Radioisotope Unit, Department of Medicine, University of Michigan Medical School, Ann Arbor. (Aided by grants from the State of Michigan and U. S. Public Health Service.)

This study was designed to compare the relative usefulness of various tests of intestinal absorption, both as screening procedures and as quantitative indices of this function. Chemical determinations of fecal fat and nitrogen during a 5-day period of measured food intake (with chemical analysis of representative diets) served as a quantitative reference standard for comparison with the other tests. A test meal containing olive oil, vitamin A, either casein or gelatin, glucose and either $I^{131}$ triolein or $I^{131}$ albumin (RISA) was used in over 100 studies in patients with widely varying absorptive status. Blood, urinary and fecal radioactivity, vitamin A and glucose tolerance curves were compared with coefficients of fat absorption. Increased fecal fat was accompanied by a parallel increase in fecal nitrogen indicating that absorption defects consistently include loss of both fat and protein. Fecal radioactivity after the test meal showed a very high coefficient of correlation with fecal fat and nitrogen in spite of a few disturbing instances where correlation was not good. Urinary radioactivity in 24 or 72 hours correlated less well than did fecal radioactivity, while vitamin A, blood radioactivity and glucose tolerance curves showed only fair correlation with fecal fat, nitrogen or radioactivity. Although most diseases of malabsorption showed very similar patterns of absorption as reflected by the tests, there were a few exceptions characteristic of specific syndromes. These included the tendency to diabetic glucose tolerance curves in pancreatic disease, a slight tendency towards higher fecal fat/nitrogen ratios in active nontropical sprue but not in secondary sprue, and elevated blood curves of all types in postgastrectomy patients even in the presence of marked steatorrhea. These systematic variations were eliminated from the calculation of coefficients of correlation in the comparison of tests.

### Radioactive Fat Absorptive Patterns in Obesity

By *Donald Berkowitz and Nathaniel Berk.* Sidney Hillman Medical Center, and Department of Medicine, Albert Einstein Medical Center, Northern Division, Philadelphia.

Recent experiences with radioactive triolein test meals have demonstrated the usefulness of this technic in the study of fat absorption in various disease states. After the oral ingestion of a measured amount of this material, characteristic blood absorptive patterns are obtained. A decreased blood radioactivity curve is seen in diseases of malabsorption such as sprue, pancreatic insufficiency and obstructive jaundice. Higher than normal levels, on the other hand, have been found in patients with coronary atherosclerosis and hypercholesterolemia, alone or in combination.

Because of the well documented relationship between obesity and cardiovascular diseases, we have performed a radioactive fat tolerance test on 25 obese subjects. In more than 50%, abnormalities have been demonstrated, the overall curve being similar to that obtained in patients with atherosclerosis of the coronary arteries—i.e., an elevated blood radioactivity concentration with abnormal retention even after 24 hours. These abnormalities were exaggerated when the obese state was complicated by diabetes and/or an elevated cholesterol value.

The data indicate that an error in fat metabolism is present in a high percentage of obese subjects which is similar to that observed in patients with coronary atherosclerosis. This establishes in a more definitive manner the relationship between these two pathologic entities.

### Comparative Effect of Parasympathomimetic Agents and 5 Hydroxytryptamine upon Colon Contractility in Dogs

By *Marvin H. Sleisenger, David H. Law, Charles M. Lewis and James H. Pert.* Department of Medicine, New York Hospital–Cornell Medical Center, New York.

Although earlier studies in man indicated that excessive cholinergic stimulation of the colon may play a role in the mechanisms of diarrhea, anticholinergic drugs have been partially or wholly ineffective in controlling diarrheal disorders. For this reason a number of factors believed to be operative in the neurohumoral stimulation of colonic motility have been systematically studied.

In anaesthetized dogs, the contractile re-

sponses of the proximal colon were recorded kymographically following intubation and placement of a small water filled latex balloon. Drugs were injected into the terminal artery of the segment under study, or on occasion intravenously. In the completion of some experiments, the colonic segment was removed and tissue cholinesterase (Che) was measured manometrically.

Similar patterns of contraction and comparable dose-response curves were obtained for acetylcholine (Ach), methacholine, and 5 hydroxytryptamine (5 HT); for all 3 agents, threshold effects were obtained with 0.00001 to 0.001 mg. After reduction of tissue Che by di-isopropylfluorophosphate (DFP) to 25% or less of normal values, the threshold doses of Ach and methacholine were not lowered, but contractions of greater amplitude and duration were produced. Atropine administered either intravenously (1.0–100.0 mg.) or intra-arterially (10.0–20.0 mg.) altered threshold response to both Ach and 5 HT raising threshold by 10 to a 100-fold. Hexamethonium, 1.0–2.0 mg./Kg. introduced intravenously or 0.1 mg./Kg. intra-arterially, failed to alter threshold of either drug; however, response to serotonin appeared to be potentiated. Intravenous doses of the benzyl analogue of 5 HT (BAS), 20.0–100.0 mg. did not affect either 5 HT or Ach response; however, 2.0–3.0 mg. given into the artery temporarily (20–30 min.) reduced sensitivity to 5 HT by a 100-fold. It had no effect on Ach threshold.

These results indicate a limited role for tissue Che in this neuroeffector system. The close similarity of Ach and 5 HT in their threshold doses, and in the modification of their patterns of response by atropine suggests that they may act synergistically in stimulating colonic motor activity.

**The Electrical Potential Developed by the Large Intestine: Its Relation to Electrolyte and Water Transport**

By *I. L. Cooperstein and Stanley K. Brockman.*

National Heart Institute, Bethesda.

The large intestine is known to reabsorb salt and water, but a lack of information about the electrical potential difference across its epithelium has prevented a precise analysis of this phenomenon. The purpose of this study was to see if an electrical potential could be measured and then to correlate it with water and electrolyte transport.

A preparation was devised in which an electrolyte solution was recirculated through a segment of a dog's large intestine, with blood supply intact. Water movement was indicated by change in concentration of phenol red. Net transfer of $Na^+$, $K^+$, $Cl^-$, and $HCO_3^-$ was determined chemically. Unidirectional movements of $Na^+$ and $Cl^-$ were determined with $Na^{24}$ and $Cl^{36}$. Simultaneously, an electrical potential difference was measured potentiometrically using 2 agar bridges in contact with the lumen and peritoneal surface.

In 13 dogs, a stable potential of 10–40 mV. was observed, lumen negative with respect to peritoneal surface (the latter being equipotential with circulating blood).

The net movement of $Na^+$ from lumen to blood was "uphill," i.e. against an electrochemical potential gradient. $HCO_3^-$ secretion into the lumen (equivalent to $OH^-$ secretion or $H^+$ reabsorption) was variable, but in some instances this ion was actively secreted. Net secretion of $K^+$ and net reabsorption of $Cl^-$ were "downhill."

Strophanthidin, a known inhibitor of cation transport in other tissues, when added to the perfusate at $3 \times 10^{-6}$ M, resulted in an immediate drop of at least 50% in the observed potential and partial inhibition of sodium and bicarbonate active transport.

Correlation of these electrical and chemical events indicates that the primary source of the observed electrical potential is active $Na^+$ reabsorption. The active secretion of $HCO_3^-$ from blood to lumen contributes a part, and in some instances a major part, in the generation of this potential difference.

# IMMUNOLOGY

### Use of the Tannic Acid Hemagglutination Test to Detect the Reaction Between L.E. Sera and Nucleoprotein Extracts or DNA

By *Howard C. Goodman and Robert Bowser.* National Heart Institute, National Institutes of Health, Bethesda.)

The demonstration that gamma globulins from the sera of patients with systemic lupus erythematosus localized on the nuclei of white blood cells (Holman and Kunkel, 1956) suggested the possibility of attempting to detect the L.E. factor with serologic methods. A low ionic strength nucleoprotein extract (Chargaff and Davidson, 1955) was made from human liver and kidney and rabbit liver obtained at autopsy. The extracts were cleared by ultracentrifugation, diluted 1:400 with 0.15 M. saline (pH 7.2) and mixed for 30 minutes at room temperature with an equal volume of a 2% suspension of tannic acid-treated human type O, Rh neg. blood cells. After washing, 0.1 ml. of extract-coated RBCs was suspended in tubes containing serial dilutions of sera to be tested, and the patterns of agglutination were read after 17 hours at 2 C. Active extracts were obtained from human liver and kidney and rabbit liver. Ten of 18 sera from patients with systemic lupus erythematosus agglutinated extract-coated RBCs in titers ranging from 1:16 to 1:1280. Forty-five control sera from patients with a variety of diseases were negative. When a 0.001% solution of calf thymus DNA was used to coat the tanned RBCs, similar results were obtained. However, sera previously absorbed with DNA continued to react with nucleoprotein-coated cells and sera absorbed with nucleoprotein extracts continued to react with DNA-coated cells. Thus at least two "antibodies" against nuclear material are demonstrable in the serum of certain patients with lupus erythematosus.

# INFECTIOUS DISEASES

### Influence of Splenectomy on Resistance to Infection in Rats

By *Harvey Rothberg and Leon A. Corallo.* Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C.

Recent clinical reports have suggested enhanced susceptibility to infection in splenectomized infants and children; however, other clinical experience does not accord with this concept. An experimental approach to this problem was made with suckling rats of a Bartonella-free CF Nelson strain. Forty-five 6 to 11-day-old rats were splenectomized, while 51 littermate controls were subjected to laparatomy. Approximately 10 days later, when animals were 17 to 20 days old, they were challenged by intraperitoneal injection of graded dilutions of a 10-hour 37° culture of highly virulent encapsulated type I pneumococci. For each dosage of inoculum, littermate controls were used. Animals were checked twice daily, and were found dead at periods varying from 48 to 96 hours after inoculation; at the higher dilutions of bacteria, there were many survivors. Postmortem examination revealed pneumococcal bacteremia in all animals who succumbed. The mean survival time of the 26 splenectomized animals who died was 60.1 hours; that of the 29 dead controls was 58.6 hours. The $LD_{50}$ (calculated by the method of Reed and Muench) for the splenectomized group was 0.33 bacteria; for the laparotomized controls, it was 0.36 bacteria. It is concluded that under the conditions of this experiment, there is no demonstrable influence of splenectomy on resistance to pneumococcal infection in young rats.

### The Effect of Penicillin on Klebsiella in the Respiratory Tract

By *William Weiss, George M. Eisenberg and Harrison F. Flippin.* Division of Bacteriology and Department of Pulmonary Diseases, Philadelphia General Hospital (Blockley Division); Section of Infectious Diseases, University of Pennsylvania.

This study was designed to determine the effect of penicillin on sputum flora in vivo and in vitro with special reference to Klebsiella species. The low capsular types of Klebsiella, 1 to 4, are uncommon but are often clearly primary respiratory tract pathogens, since the organisms are already in the respiratory tract at the time that the patient is admitted to the hospital, and their

presence is frequently associated with destructive lung disease. In contrast Klebsiellae of the higher types are much more common in the sputum of hospitalized patients but usually appear some time after admission and seldom seem to play a primary pathogenic role. They are found as often in patients who are not receiving antibiotics as in those who are.

It has been postulated that gram-negative bacilli exist in the respiratory tract in unnoticed small numbers and become the predominant organisms when the indigenous respiratory bacteria are inhibited by antibiotics. With particular reference to penicillin and Klebsiella, this hypothesis is not supported by two experiments: (1) the administration of penicillin in usual dosage to 14 patients failed to convert the sputum flora to one in which gram-negative bacilli predominated; and (2) penicillin in vitro usually failed to reveal gram-negative bacilli in sputa when they were not present in routine cultures.

The above considerations suggest that the frequent appearance of the higher types of Klebsiella species in the respiratory tract of hospitalized patients may be due more to nosocomial spread than to a direct effect of antibiotic therapy upon the microflora.

### Needle Biopsy of the Parietal Pleura in Tuberculous Effusion

By *William Weiss*. Department of Pulmonary Diseases, Philadelphia General Hospital, Philadelphia.

This study was undertaken to compare the results of needle biopsy of the parietal pleura and cultures of pleural fluid for tubercle bacilli in a series of patients with tuberculous pleural effusion. Needle biopsy was done in 31 cases with the Vim-Silverman needle. Caseating tubercles were demonstrated in 74% of the cases, whereas pleural fluid cultures were positive in 44%. Tuberculosis was established as the etiology of the pleural effusion in 87% by all methods. Only 4 cases remained "idiopathic" and were treated as tuberculous in the presence of a positive tuberculin test. Needle biopsy was the single most rewarding diagnostic tool. It is a simple convenient procedure which is innocuous and repeatable.

### A New Bactericidal Antibiotic, Vancomycin, in the Treatment of Micrococcal Endocarditis

By *Joseph E. Geraci, Fordyce R. Heilman, Donald R. Nichols and William E. Wellman*. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

During the past 20 months we administered vancomycin as the sole therapeutic agent to 6 patients with endocarditis due to coagulase-positive micrococci. Four patients were men and two were women. The average age was 48 years (range 20 to 71). The average duration of the infection before treatment was 3 weeks (range 1 to 6 weeks). The sources of infection were: ulcerative lesion of the extremity or back, 3; infection of urinary tract (postoperative), 1; and no demonstrable source, 2. The duration of therapy ranged from 2 to 4 weeks. Vancomycin, 0.5 Gm., was given intravenously every 6 hours over a period of 3 to 4 minutes.

Four patients were cured, and one patient died after 5½ days of therapy, with infection uncontrolled. Another patient died 3 weeks after therapy was concluded, the infection controlled, the lesions healing, and all cultures negative. Both deaths were from congestive heart failure. The remaining 4 patients have had follow-up periods of 2 to 20 months. Serum levels of vancomycin with daily doses of 2 Gm., in the presence of good renal function, averaged 13 ± μg./ml. (range 7.6 to 17.2). In one patient with impaired renal function serum levels reached 80 to 95 μg./ml. In all 6 patients therapy was guided by serum bactericidal tests, the patient's serum being tested with the patient's organism. The total killing effect was noted in a serum dilution of 1:8 in 5 patients and a dilution of 1:4 in one patient. Toxicity from vancomycin consisted of phlebitis of varying degree in all 6 patients, skin rash, and moderate deafness in one patient (patient with renal insufficiency and high serum levels), and possibly drug fever in another patient.

These preliminary experiences indicate that penicillin-resistant micrococcal endocarditis may be treated successfully with vancomycin.

### Treatment of Staphylococcal and Gram-negative Bacillary Infections with Kanamycin

By *Paul A. Bunn and Aldona Baltch*. Department of Medicine, State University of New York, Upstate Medical Center, Syracuse. (Aided by a grant from Bristol Laboratories, Syracuse.)

In vitro observations indicate that Kanamycin inhibits the growth of penicillin-resistant staphylococci, most gram-negative bacteria and mycobacterium tuberculosis. A small series of patients with acute bacterial and coccal infec-

tions have been treated with it, and this report describes preliminary observations about its clinical efficacy. As the agent is quickly absorbed from an intramuscular depot only, all patients received it by that parenteral route. The usual dosage was 0.5 Gm. twice daily. Toxicity was not observed in any patient. The duration of therapy extended from 3 to 17 days.

Eight patients with serious staphylococcal disease were treated with Kanamycin; in 5 it was the sole therapeutic agent. Failure of other drug therapy had been experienced in 5. Positive blood cultures were present in 3 at the time therapy was started. Diagnosis included carbunculosis, pneumonia, endocarditis, purulent pericarditis and postoperative wound infections.

Response to therapy in 6 was impressive. In one patient concomitant use of penicillin for a penicillin-susceptible staphylococcal endocarditis made interpretation difficult, but the patient recovered. Another patient with acute pneumonia died after 4 doses—autopsy revealed lungs highly infected with staphylococci.

Seven patients with gram-negative bacillary infections were treated. Six had acute genitourinary tract infections, the other lobular pneumonia. Either proteus or coliform organisms were isolated from all; 3 had positive blood cultures when treatment was initiated.

Responses in 4 were satisfactory including impressive improvement in 2 of the 3 with bacteremia. Responses in the remaining 3 were difficult of evaluation; 2 died from other causes shortly after starting treatment.

There is sufficient experience with Kanamycin to suggest that it has a place in the therapy of acute infections caused by staphylococci and gram-negative bacilli resistant to other commonly used antimicrobial agents. The drug warrants broader study.

### Cranberry Juice and the Antibacterial Action of Hippuric Acid

By *Phyllis T. Bodel, Ramzi Cotran and Edward H. Kass.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, Department of Medicine, Harvard Medical School, and Mallory Institute of Pathology, Boston City Hospital, Boston.

Cranberry juice is frequently used pragmatically in infections of the urinary tract, but objective data concerning its effectiveness or possible modes of action are not available. It has been established that cranberries and cranberry juice contain quinic acid which is excreted in the urine as hippuric acid. When large amounts of cranberries are fed, slight systemic acidosis may occur and there is a marked increase in urinary excretion of hydrogen ions.

Observations with hippuric acid in vitro showed that at pH 5.0 cultures of *E. coli* or of P. vulgaris were sterilized by 0.025 M. hippuric acid, whereas this pH was not lethal in the absence of hippuric acid. The bacteriostatic concentration of p-aminohippuric acid at pH 5.0 was 0.1 M. The bacteriostatic action of hippuric acid at different pH values was shown to be a direct function of the number of unionized molecules of the organic acid in solution.

When 600 ml. of cranberry juice (which has a pH of 2.5–3.0) were fed to normal adults, the hippuric acid concentration in the urine was in the range of 0.01–0.02 M., a level marginally adequate for bacteriostasis. The pH of the urine fell but slightly.

However, when 12–15 Gm. of sodium benzoate or of hippuric acid were fed to adult patients with chronic infections of the urinary tract, the pH of the urine was frequently maintained at 5.0 and bacteriuria was reduced or eliminated even in patients whose infections of the urinary tract were resistant to previous antibiotic treatment.

Although the mechanism of action of hippuric acid is unclear, its antibacterial action is a function of pH, it is relatively nontoxic, and it serves in the renal tubule to increase the excretion of hydrogen ions. These considerations have been put to therapeutic use; they also suggest a pathway for future investigation of antibacterial agents.

### The Influence of Triiodothyronine on the Lethal Effect of Bacterial Endotoxin and Infection Due to *Br. melitensis*

By *James C. Melby, G. Mary Bradley and Wesley W. Spink.* Department of Medicine, University of Minnesota Medical School, Minneapolis.

Thyroid hormone accelerates the allergic reaction and increases the natural resistance to bacterial infection in some species. These studies were undertaken to assess the influence of triiodothyronine on the lethal effect of brucella endotoxin and the resistance to infection with *Brucella melitensis* in mice.

Intraperitoneal injections of triiodothyronine were given at 24 and 6 hours prior to the injection of endotoxin. Ninety % of the control animals

survived 18 hours or more, whereas only 20% of the group which received triiodothyronine survived this period.

Mice pretreated with triiodothyronine for 4 days were infected with *Br. melitensis* intraperitoneally and sacrificed after 14 days of additional treatment. Quantitative spleen counts of *Br. melitensis* were less following small doses of bacteria, but not significantly different from controls given larger doses.

Infected animals treated with large doses of triiodothyronine exhibited unique destructive lesions of the liver. These lesions were not found in the infected control animals or animals treated only with triiodothyronine.

These studies indicate: (1) that triiodothyronine significantly enhances the lethal effect of endotoxin; (2) that the protective effect of triiodothyronine against *Br. melitensis* is dependent upon the inoculum of brucella; (3) large doses of triiodothyronine administered to mice infected with *Br. melitensis* result in a unique type of hepatic lesion unlike the classical granulomatous lesion of brucellosis.

**A Test of the Hypothesis of Actively Acquired Tolerance in the Human Indicated by Immunity to the Poliomyelitis Virus**

By *David C. Poskanzer and William G. Beadenkopf.* Communicable Disease Center, Public Health Service, and the New York State Department of Health.

The theory that exposure to an antigen prior to the maturation of an animal's antibody-producing mechanism might result in inability of the animal to produce antibodies to that antigen later in life was proposed by Burnet and Fenner in 1949. This phenomenon, termed actively acquired tolerance, has not been tested in the human subject. This investigation attempted to utilize transplacental virus infection with poliomyelitis virus to detect tolerance in 12-year-old children whose mothers had paralytic poliomyelitis when pregnant. Twelve mothers were located who had had paralytic poliomyelitis illness during 1944, an epidemic year in New York State. Onsets were distributed through the 9 months of gestation. Stimulation of the antibody-producing mechanism in their 12-year-old offspring was accomplished by the use of Salk poliomyelitis vaccine.

Sera were obtained from mothers and their 12-year-old children. These sera were tested for antibodies to poliomyelitis virus by standard tissue culture tests.

It was shown that in 11 of the 12 instances, antibodies were readily produced against all 3 types of poliomyelitis virus. In one instance, antibody was not so produced to Type III poliomyelitis virus.

Thus, actively acquired tolerance to poliomyelitis virus could not be detected in this small group of children. Several alternative explanations might be offered for these results: (1) tolerance may develop during a very brief period of intrauterine life not detectable in this group; (2) interference with production of antibodies may have been partial rather than complete, and overcome by the antigen employed; (3) in maternal infection with poliomyelitis virus, transplacental infection or exposure of the fetus may be a rare phenomenon; (4) tolerance may not be acquired to the poliomyelitis virus particle.

**Prevention of Rubella with Gamma Globulin**

By *Harold B. Houser and Norbert Schalet.* Laboratory on Housing and Illness, Armed Forces Epidemiological Board and Department of Medicine, State University of New York, Upstate Medical Center, Syracuse. (Aided by Commission on Acute Respiratory Diseases, AFEB and Surgeons General of the Army and Air Force.)

During a study of the effect of gamma globulin on prevention of respiratory disease in Air Force recruits, the occurrence of epidemic rubella enabled us to make observations on its prevention.

Two groups of men received either 5 or 15 cc. of gamma globulin. A 3rd group was given saline. Injections were given within 24 hours of the men's arrival at Sampson Air Force Base. All men in a 60-man flight received the same injection. Each group was composed of 5 flights (300 men). Benzathine penicillin, 600,000 units, was given intramuscularly initially and at 4 weeks unless contraindicated. Complete sick-call records were maintained and examination of all hospital admissions was made. Serum was obtained from each man at 0, 6, and 10 weeks, the period of observation. In addition, hospital admissions from the remainder of the base were screened for rubella diagnoses.

Total hospital admissions from the saline, 5 and 15 cc. gamma globulin groups were 29, 10, and 11, respectively. Of the 50 admissions, 27 were for nonstreptococcal respiratory disease, 14 for streptococcal infections, and 9 for rubella.

Seven of the rubella admissions were from the saline group and the remaining 2 were from the 5 cc. gamma globulin group. No admissions for rubella occurred in the 15 cc. gamma globulin group. During the study, 173 cases of rubella were admitted to the hospital from the remainder of the base population. 108 of these occurred during the 4 weeks when rubella was present in the study groups. The pattern of sick-call attendance in the 3 groups was similar to that of hospital admissions.

It is concluded that gamma globulin, given before exposure, prevented rubella. Reduction in sick call and nonrubella admission might have been the result of the prevalence of rubella without rash.

### A Study of Fatal Cases of Asian Influenza

By *Robert Oseasohn, Lester Adelson, Masaro Kaji and David Stevens.* Department of Preventive Medicine, Western Reserve University, and Cuyahoga County Coroner's Office, Cleveland.

The recent epidemic of Asian influenza has been associated with a high attack rate and excess mortality. Fulminating illnesses, similar to those reported in the pandemic of 1918–20, have been observed. Studies were performed on 43 influenza-associated deaths, 25 of which were confirmed by isolation of Asian virus.

The cases were diagnosed as influenza clinically and pathologically, or, in unsuspected instances, from morphologic changes. Suspensions of lung, trachea and other tissues were utilized for viral and bacterial isolation. In 5 cases from which virus was not isolated, infection was indicated serologically.

Clinically, 18 cases were characterized by a fulminating course and death in less than 4 days. Of these, 8 had complications including heart disease, encephalopathy and pregnancy. Antibiotics were administered in 12 of these cases, 3 of which had evidence of bacterial pneumonia. One of the 6 untreated cases had pneumococcal pneumonia. The other 14 cases had no gross evidence of bacterial infection. Of 3 other cases, 2 collapsed and died suddenly, and 1 died 3 days after head injury; neither had clinical evidence of influenza. In the remaining 22 cases death occurred from 5 to 10 or more days after onset. Twelve had complicating conditions. Antibiotics were given in 11, 3 of which showed gross evidence of bacterial pneumonia. Of 11 untreated cases, 2 had bacterial pneumonia.

The cases were distributed in all age groups; 13 occurred in persons under 20; 14 in persons 40–59. Fulminant cases were characterized by wet, hemorrhagic lungs, and necrotizing tracheobronchitis, and in 2 instances by staphylococcal pneumonia and abscess formation. Cases of longer duration were usually characterized by pulmonary consolidation.

This study indicates that many of the features of deaths assocaited with the recent Asian influenza epidemic are similar to those of the pandemic of 1918–20.

### Clinical and Pathologic Studies in Severe Asian Influenza Infections

By *Fred R. McCrumb, Jr., Paul F. Guerin, George K. Baer and Theodore E. Woodward.* Departments of Medicine and Pathology, University of Maryland, School of Medicine, Baltimore.

The pulmonary manifestations of human influenza A infections as well as cardiovascular abnormalities associated with this disease have been described. In most instances pulmonary complications were attributable to superimposed bacterial infections. Among several hundred patients who were studied by our group in Baltimore during the recent Asian influenza epidemic, 15 were encountered whose illness was unusually severe. In most instances, the disease was complicated by pneumonia with or without evidence of cardiovascular derangement. In all but one patient evidence of infection with influenza virus, Asian strain, was obtained by isolation of virus or the demonstration of specific antibody. Nine of these patients who succumbed to the disease provided additional material for virologic and bacteriologic study.

Six patients developed roentgen evidence of pneumonitis several days after the onset of an acute respiratory disease resembling influenza. Influenza virus, Asian strain, was isolated from one patient and all developed hemagglutination-inhibiting (HI) antibody during the illness. Sputum and throat cultures revealed a predominance of *Diplococcus pneumoniae*. Among the fatal cases, Asian influenza virus was isolated from lung tissue in 3 instances. The remaining 6 patients had varying amounts of HI antibody at the time of death and, in the absence of a history of previous respiratory illness or vaccination with Asian virus, this was assumed to be related to the illness in question. In most instances, pneumonia was thought to be bacterial on the basis of histologic study and isolation of *Micrococcus pyogenes* or *D. pneumoniae* from involved lung

The presence of interstitial reaction and hyaline membrane formation in some specimens suggests that pneumonitis may be initiated by Asian influenza virus. Electrocardiographic evidence of myocardial or pericardial disease was obtained in two patients and histologic evidence of myocarditis was observed in one of the fatal cases.

### Comparison of Clinical Manifestations of Respiratory Illness Proven to be Due to Asian Influenza, Adenovirus, and Unknown Cause

By *Irwin Schultz and Benjamin Gundelfinger.* Naval Medical Research Unit No. 4, Great Lakes, Illinois.

Recent advances in laboratory technics have aided in the etiologic classification of acute respiratory diseases of military recruits, and have provided information on the pathogenesis and clinical manifestations of these common illnesses.

A specific diagnosis could be made or reasonably excluded by standard virologic and serologic methods in over 200 naval recruits hospitalized during a 4-month period when Asian strain influenza and adenovirus infection were both frequently associated with febrile respiratory illness, and streptococcal carrier rates were low.

Significant differences ($p < .05$) in clinical manifestations between those illnesses associated with influenza and those with adenovirus infections were observed. The clinical finding of the influenza group were characterized by sudden onset, headache and predominantly constitutional manifestations. The adenovirus group had a significantly higher incidence of signs and symptoms referable to the throat such as soreness, dysphagia, hoarseness, and lympoid hyperplasia. The greatest difficulty in differentiation of the 2 groups occurred in those cases with predominantly lower respiratory tract involvement representing about 1/3 of all cases in which a specific etiologic diagnosis was possible. Excluding these, an accurate diagnosis could have been made from the clinical findings alone in over 50% of the remaining cases. Those cases which were found to have neither influenza nor adenovirus infection did not manifest distinguishing clinical findings. Pneumonitis, as diagnosed clinically or by x-ray examinations, represented about 10% of the cases and was found in all groups with about equal frequency.

These findings indicate the importance of adenovirus as a cause of pharyngitis in military recruits in the absence of streptococcal infection. The manifestations of Asian influenza did not appear to differ from those associated with other influenza strains. Differences in clinical manifestations could be helpful in distinguishing cases due to influenza and adenovirus as seen in a recruit population.

## KIDNEY

### A Study of a Protein Analog of Biologic Membranes

By *George J. Brewer and John D. Arnold.* University of Chicago.

A limiting factor in many biologic and clinical studies is the lack of a proper dialyzing membrane. An exploration of the basic properties of protein membranes was undertaken with the view of filling this need with membranes as nearly analagous to functioning biologic membranes as possible. Fibrinogen has been evaluated because it is relatively easy to obtain from the animal of choice. Using this substance it was possible to obtain an organized array of biologic polymer in the form of a membrane approximately 100 A in thickness. This approaches the probable thickness of the basement membrane of the glomerulus, and we believe marks the first time that an artificially synthesized membrane of this order of magnitude and composed of a biologic substance has been produced.

The technic involves dipping a properly constructed grid of quartz fibers through a monomolecular film of fibrinogen at an air-water interface. A continuous bimolecular film is deposited, and if desired, a polymolecular film can be built by repeated dipping. It is also possible to add other biologic molecules, such as lipids, lipoproteins, or other proteins.

Results demonstrate that these ultra-thin membranes are remarkably stable when kept in aqueous solution. The membranes act as a diffusion barrier or molecular filter with retardation dependent upon solute molecular weight. The dialyzing characteristics of fibrinogen membranes have been worked out. Further, the structure of the membranes has been demonstrated microscopically.

A stable biologic dialyzing membrane has

been developed, and it is our conclusion that this membrane comes closer than any previously described synthesized membrane to duplicating glomerular capillary basement membranes in thickness, function and composition.

### Renal Blood Flow Determined by the Use of Radioiodinated Serum Albumin and an Externally Placed Scintillation Detector

By *Gunnar Sevelius and Philip C. Johnson.* Radioisotope Service, V. A. Hospital, and Department of Medicine, University of Oklahoma, Oklahoma City.

Cardiac output can be estimated by using a scintillation detector placed over the heart to measure the passage of radioactive serum albumin. We have found that this technic can be used to estimate kidney blood flow by comparing the ratio of the area of the curve obtained during one circulation time over each kidney to the area of the heart curve during this same time. In 7 patients without renal or cardiac disease the mean cardiac blood flow (CBF) was 4618 ± 191 cc./min. (mean ± S.E.), the right kidney blood flow (RKBF) was 715 ± 47 cc./min. and left kidney blood flow (LKBF) was 689 ± 22 cc./min. In a patient with unilateral kidney disease CBF was 4686, RKBF was 1667, and LKBF was 00. A twin, 2 months after a kidney transplant, had a CBF of 8400 and transplanted kidney blood flow of 1714. This kidney had a normal blood flow before the transplant. A patient with chronic glomerulonephritis with uremia had a CBF of 2625, RKBF of 320 and LKBF of 311. A patient with intracapillary glomerulo sclerosis had a CBF of 3367, with a RKBF 204 and a LKBF of 265.

It would seem that this technic offers a simple method for determination of renal blood flow in normal patients and in patients with kidney disease.

### Renal Clearance of $I^{131}$-Diotrast at Low Plasma Concentrations

By *Jerome B. Block and Belton A. Burrows.* Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals; Radioisotope Service, Boston V. A. Hospital; and Departments of Medicine, Boston University School of Medicine, Boston. (Aided by an A.E.C. contract.)

We have found that the 30-minute excretion of a tracer dose (<1 mg.) of $I^{131}$-Diodrast by normal subjects is doubled if preceded by a small dose (75 mg.) of carrier Diodrast. The diminished excretion of $I^{131}$-Diodrast given alone may be a manifestation of both hepatic-binding and reduced renal clearance of $I^{131}$-Diodrast at low plasma concentrations. Renal clearance of $I^{131}$-Diodrast and the effects of carrier Diodrast were studied at plasma concentrations below 10 μg./100 ml. of plasma (gamma %), calculated from plasma radioactivity and specific activity of dose.

Following a single injection of $I^{131}$-Diodrast there was a rapid fall in plasma concentration. After 20 minutes, however, plasma disappearance was slower than anticipated from the reported extraction ratios for Diodrast. Clearance of $I^{131}$-Diodrast during this later period, at plasma levels below 0.5 gamma %, was in the range of 200–250 cc./min. Continuous infusion of $I^{131}$-Diodrast at plasma concentrations below 0.5 gamma % yielded similar clearances. Subsequent addition of carrier Diodrast had no effect on clearance or plasma levels of $I^{131}$-Diodrast.

Infusion of $I^{131}$-Diodrast at rates to maintain plasma concentrations above 1.0 gamma %, resulted in clearances of 600–700 cc./min. At plasma concentrations between 0.5 and 1.0 gamma % intermediate clearance values were obtained. When carrier Diodrast was infused at rates below tubular maximum secretory capacity (Tm), before and with $I^{131}$-Diodrast, clearances of approximately 600 cc./min. were seen with plasma $I^{131}$-Diodrast levels below 0.5 gamma %. Infusion of carrier Diodrast above Tm resulted in lower $I^{131}$-Diodrast clearance.

These studies indicate that Diodrast clearances are reduced not only at plasma Diodrast levels above Tm, but also at low plasma levels obtained with $I^{131}$-Diodrast alone. This may be the result of strong plasma-binding of small amounts of $I^{131}$-Diodrast which reduces the availability of $I^{131}$-Diodrast for secretion by the renal tubular cell.

### The Relationship between Net Water Reabsorption and Osmolal Clearance as a Measure of Renal-Concentrating Activity

By *Lawrence G. Raisz, William Y. W. Au and Robert L. Scheer.* Medical Research Laboratory, V. A. Hospital, and Department of Medicine, State University of New York Upstate Medical Center, Syracuse.

The relationship between net water reabsorption ($T^cH_2O$) and osmolal clearance ($C_{osm}$) has been used as a measure of the response of the renal concentrating mechanism to varying

solute loads. In the present study this relationship has been evaluated over a wide range of solute excretion in normal young men deprived of water for 18–24 hours. Solute loading was generally accomplished with 5% mannitol solution, but data were also obtained following loading with urea and with hypertonic mannitol solution. 200 to 600 mu./hr. of vasopressin were given with the infusions.

As $C_{osm}$ was increased up to 20–30 ml./min./1.73 M.², $T^cH_2O$ showed a continuous curvilinear increase and did not reach a constant value. The data obtained at steady, moderately increasing, and moderately decreasing rates of solute excretion were similar, but when $C_{osm}$ increased rapidly, the values obtained for $T^cH_2O$ were often aberrantly high. The effect of hypertonic mannitol or urea-loading was similar to that of isotonic mannitol-loading. The curve relating $T^cH_2O$ and $C_{osm}$ was reproducible in different experiments on the same subject and could be employed to evaluate changes in concentrating ability at intermediate rates of solute excretion.

When $C_{osm}$ was increased from 20 to 40–50 ml./min./1.73 M.² a further increase in $T^cH_2O$ was observed in one subject, but in 4 subjects $T^cH_2O$ decreased from values of 3.2–5.2 to 0.1–2.8 ml./min./1.73 M.² In none of the experiments was $T^cH_2O$ constant. The decrease in $T^cH_2O$ at high rates of solute excretion indicates either that concentrating activity was diminished by large isotonic mannitol loads, or that the fluid delivered to the concentrating site may have become hypotonic so that $T^cH_2O$ did not measure the rate of water reabsorption at this site.

### Effect of Protein-feeding on Urine Concentration: Suggestive Evidence of Tubular Secretion of Urea

By *Milton E. Rubini and William B. Blythe.* Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C.

How urea augments urine-concentrating ability is unknown. When urea is given chronically (or fed as high-protein dietary equivalent) water conservation is enhanced in normal dogs and men, and in patients with hyposthenuria. When given acutely during hydropenia, the osmolar urine/plasma gradient is not increased in normal men, but is increased in some patients with renal disease. Maximum water reabsorption ($T^cH_2O$) during mannitol diuresis, although enhanced by antecedent high-protein diet, is not increased if urea is acutely given to man, but is regularly increased in dogs. These inconsistencies may be due to the influence of antecedent diet, whereby some adaptation of tubular function may cause an apparent increase in water reabsorption.

Urea was given during hydropenia and at the height of mannitol diuresis to normal men prepared by prefeeding a constant diet containing similar water and total solute, but varying in protein. At wide range of urine flow and solute excretion, urine became more concentrated after urea with high-protein prefeeding, but did not when protein was restricted. Dogs did not increase $T^cH_2O$ after urea if they were prefed a low-protein diet. In both men and dogs the clearance ratio of urea to creatinine increased more rapidly during mannitol diuresis after high-protein feeding, and at low and intermediate urine flow was significantly affected by antecedent diet. The increment of water reabsorbed could in each instance be accounted by the amount of urea present, if it is assumed that $< 50\%$ of the urea obligated no water for excretion.

Such data indicate that apparent increased concentrating ability after brief high-protein feeding may be ascribed to increased excretion of urea presumably by some process of tubular adaptation whereby urea excretion, independent of, or distal to water reabsorption may continue within the limiting osmotic ceiling of other urinary solute.

### The Effects of Hypokalemia and Hypercalcemia on the Renal-concentrating Mechanism

By *W. G. Walker and C. C. J. Carpenter.* Johns Hopkins Hospital, and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore.

The marked polyuria frequently associated with instances of hypokalemia and hypercalcemia has not been adequately explained. Using cryoscopy to measure osmolal concentrations in blood and urine, and endogenous creatinine clearance as a measure of GFR, the renal concentrating mechanism has been studied in patients exhibiting these disturbances.

Responses to intravenous pitressin and an osmotic load of mannitol were studied in 2 patients with severe potassium depletion (initial serum K concentrations less than 2.0 mEq./L.) While depleted, both patients elaborated urine significantly hypotonic to plasma (urine Osm. 177 and 255 mOsm./Kg. urine $H_2O$, respectively), despite fluid restriction, pitressin and an osmotic

load of mannitol. Following small oral doses of K(50 mEq. KCl), both patients exhibited a prompt significant increase in urine osmolalities (urine Osm. 200 and 433 mOsm./Kg. $H_2O$, respectively). Within 3 to 4 days after K repletion was complete, the response to pitressin and osmotic-loading was normal as judged by a normal free water clearance [free water clearance = urine flow(V) − osmolal clearance ($U_{osm}V/P_{osm}$)].

Similar studies performed on a patient with marked hypercalcemia (serum Ca = 18 mg.%) associated with multiple myeloma showed a markedly reduced ability to conserve water. Free water clearance was only 0.56 ml./min. Four days following return of the serum Ca to normal as a result of cortisone therapy, free water clearance was 4.2 ml./min. A second patient with hypercalcemia and associated polyuria due to hyperparathyroidism was unresponsive to pitressin. He experienced prompt relief from his polyuria following operation. Studies performed at time of 6-month follow-up revealed a normal renal-concentrating mechanism.

These studies suggest that the polyuria seen in hypokalemic and hypercalcemic states may possibly be explained by unresponsiveness or diminished responsiveness of the kidney to the antidiuretic hormone.

**Changes in Electrolyte Composition of Renal Papillae in Potassium-deficient Rats Having a Urinary-concentrating Defect**

By *Malcolm A. Holliday, Thomas J. Egan and Patricia Wirth.* University of Pittsburgh School of Medicine, Pittsburgh. (Aided by a grant from the United States Public Health Service.)

Diminished ability to concentrate urine has been frequently reported, and recently this defect has been correlated with a lesion in the collecting tubules. Sodium concentration of renal papillae has been demonstrated to be greatly hypertonic to that of plasma and is correlated with ability to concentrate urine. The present study compares renal papillae sodium and potassium concentrations of potassium- deficient rats to those of controls.

The experimental group received a basal electrolyte deficient diet and 50 cc./day of a drinking solution containing 80 mM/L. $NaHCO_3$ and 20 mM/L. $NH_4CL$ for 28–33 days. The controls were on a similar regimen except that potassium chloride was substituted for ammonium chloride in the drinking solution. Maximal urinary concentrating ability was measured at the end of this period and serum, muscle and renal papillae were obtained to determine electrolyte composition. Kidney weight and histologic sections were also obtained.

In the potassium-deficient group with impaired concentrating ability renal papillae potassium per 100 Gm. of dry tissue was lower than that observed in the control group. The papillae sodium expressed as its concentration in total papillae water was significantly hypertonic to plasma in both the control and experimental group, but less so in the latter.

These data provide evidence that renal papillae cells share the potassium depletion observed in muscle cells. The observation regarding papillae sodium is in accord with previous results and demonstrates that in potassium deficiency the impaired ability to concentrate urine is correlated with a less hypertonic concentration of papillae sodium. We interpret this to indicate that impairment of urinary concentrating ability is not likely to be dependent on impairment in water diffusion through the swollen cells of the collecting tubules, but rather on some process interfering with the achievement of hypertonic fluid in the renal medulla.

**Failure of Potassium Deficiency to Induce Susceptibility to Renal Infection: An Experimental and Autopsy Study.**

By *Frank A. Carone, Michael Kashgarian and Franklin H. Epstein.* Departments of Internal Medicine and Pathology, Yale University School of Medicine, New Haven.

Both rats and mice on a potassium-deficient dietary regimen for 3 to 8 weeks failed to develop renal infection according to bacteriologic and histologic criteria when injected intravenously with a strain of E. Coli which regularly produced infection in the obstructed rat kidney. Kidneys from animals deficient for periods longer than 4 weeks showed irregular fibrosis and mononuclear cell accumulations in the medulla and cortex which, although suggestive of pyelonephritis, in view of negative bacteriologic data were interpreted as structural changes due to obstruction of the collecting tubules, in the outer medulla.

Fifteen autopsied patients, with potassium deficiency for estimated periods of 1 to 9 months, lacked evidence of acute renal infection, though active infection existed elsewhere in each case but one, terminally. Small, irregular, predominantly subcapsular scars were present in 4 kidneys, mainly from older individuals, and other

criteria of chronic pyelonephritis were absent. Vacuolar swelling of proximal convoluted tubular epithelium was present in 3 instances, a change not seen in the present experimental observations in animals but ascribed by others to potassium depletion in humans. Two of these patients had ulcerative colitis while the 3rd with hepatic failure had markedly deranged serum electrolytes due to glutamate administration. These findings suggest that this lesion is not specific for potassium deficiency.

These observations suggest that potassium deficiency of mild degree in humans and of severe degree and short duration in experimental animals may not alter the susceptibility of the kidney to infection. Deficiency of potassium appears to induce renal fibrosis which may be secondary to obstructive tubular phenomena.

**The Effect of Potassium Repletion on the Renal-concentrating Defect, the Renal Structural Changes, and the Cardiac and Skeletal Muscle Lesions Produced by Potassium Depletion in Rats**

By *Walter Hollander, Jr., Robert W. Winters, John Bradley, T. Franklin Williams, William E. Loring, Jean Oliver and Louis G. Welt.* Departments of Medicine and Pediatrics, University of North Carolina School of Medicine, Chapel Hill, North Carolina; Renal Research Unit, Overlook Hospital, Summit, New Jersey; and Department of Pathology, New York University School of Medicine, New York.

The reversibility of the renal concentrating defect and the renal structural abnormalities associated with potassium depletion in rats has been studied: (1) during 8 days immediately following rapid potassium repletion and; (2) after a prolonged (7–8 month) period of potassium repletion. In the latter study, reversibility of cardiac and skeletal muscle lesions was also examined.

Potassium depletion was produced with a potassium-free intake plus sodium salts. Renal-concentrating power was studied with exogenous vasopressin-in-oil as in previous experiments. Rapid repletion was accomplished with frequent gavage feedings of potassium chloride during 36 hours; prolonged repletion by a stock diet for 7–8 months.

From the beginning of acute repletion: (a) the concentration of potassium in dry fat-free skeletal muscle returned to normal within 2 days; (b) the renal-concentrating defect subsided rapidly and was no longer statistically significant within 3 days; (c) the granule-droplets in the collecting tubules of the inner medulla and papilla almost disappeared within 2 days; (d) the proliferative lesion in the collecting tubules of the outer medulla subsided only slightly during 8 days.

After prolonged repletion: (a) the concentration of potassium in dry fat-free skeletal muscle was the same as that of controls; (b) renal concentrating ability was normal; (c) the blood urea nitrogen was not elevated: (d) kidney tissue was bacteriologically sterile; (e) there were no lesions in cardiac or skeletal muscle; (f) the acute lesions in the renal collecting ducts had disappeared, but there were fibrous scars and cystic dilatation of collecting tubules not present in controls. Although this residual damage in the kidneys was less severe than that reported by Fourman, McCance and Parker in similar experiments, this study supports the contention that some permanent renal damage may result from an acute episode of potassium depletion.

**Renal Tubular Reabsorption of Glucose and the Mechanism of Glucosuria in Pregnancy**

By *George W. Welsh, III, and Ethan A. H. Sims.* Department of Medicine, University of Vermont College of Medicine. (Aided by a grant from the National Institutes of Health.)

To investigate the effect of pregnancy on the renal tubular reabsorption of glucose and the mechanism of the glucosuria commonly seen in pregnancy, the glomerular filtration rate (GFR), measured by inulin clearance, and the maximal tubular reabsorption of glucose ($Tm_G$) were determined in 17 normal pregnant women and 11 pregnant glucosuric women who had normal carbohydrate tolerance. 15 nonpregnant normal women served as controls.

There was no significant difference in the mean $Tm_G$ between the normal pregnant women ($378 \pm 82$ mg./min.) and the nonpregnant controls ($366 \pm 61$). The mean $Tm_G$ of the pregnant glucosuric women ($320 \pm 72$) was, however, lower than that in the normal pregnant women with glucosuria ($p < 0.05$). Moreover, in those glucosuric patients with filtration rates below the mean of $151 \pm 19$ ml./min. for the normal pregnant group, the average $Tm_G$ was significantly lower ($261 \pm 30$ with $p < 0.02$) than in both the normal pregnant women and the controls. In those with filtration rates above the mean, the average $Tm_G$ was not significantly dif-

ferent from either the normal pregnant women or the controls.

The ratio $GFR/Tm_G$ was significantly higher in the normal pregnant group ($0.41 \pm 0.08$)) than in the control group ($0.34 \pm 0.05$ with $p < 0.01$). In the glucosuric pregnant group this disproportion was more pronounced ($0.50 \pm 0.06$).

These results indicate that in spite of the increase in glucose filtered there is no increase in the absolute capacity for its reabsorption by the renal tubule during pregnancy. In many glucosuric pregnant women there is in fact a decrease, and the disproportion between filtration and reabsorption ($GFR/Tm_G$) is more marked. In the group of glucosuric patients with filtration rates above the mean for pregnancy the glucosuria appeared to be due primarily to the increase in the filtered glucose. On the other hand, in those whose filtration rates were below the mean, a significantly reduced reabsorption of glucose by the tubule would appear to be the chief factor in the production of glucosuria.

These changes in GFR and $Tm_G$ are similar in direction to those produced by the administration of 11-oxy adrenocortical steroids. This suggests that their increased secretion in pregnancy may mediate in part the observed changes.

### The Role of Transaminase in the Renal Production of Ammonia

By *Richard M. Portwood and Leonard L. Madison.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

The importance of glutaminase, glycine oxidase, and 1-amino acid oxidase in the renal production of ammonia has been clearly demonstrated in the rat. Additional ammonia production could result from transamination; both the glutamic-oxaloacetic and glutamic-pyruvic systems lead to production of glutamic acid which can be deaminated by glutamic dehydrogenase, thereby producing ammonia.

Inasmuch as the transaminating enzymes are pyridoxine dependent, it was thought of interest to determine the effect of pyridoxine deficiency on urinary ammonia excretion in normal and acidotic rats.

Obvious pyridoxine deficiency was produced in 12 rats by feeding a pyridoxine-free diet + 100 mg. of desoxypridoxine/day. After the appearance of gross signs of deficiency, 6 rats were made acidotic by the addition to their diet of 5 mM $NH_4Cl$/day. Daily 24-hour $NH_4^+$ excretion was determined for each group for 14 days and the results compared with those obtained in rats fed an identical diet which was supplemented with pyridoxine.

In the $B_6$ deficient group the mean daily $NH_4^+$ excretion was $2.76 \pm .50$ mEq./24 hrs. (control $= 2.35 \pm .23$, $p > .10$); in the $B_6$ deficient group given the acid loads a value of $7.25 \pm .61$ (control $= 6.62 \pm .46$ $p > .05$) was observed. Renal glutaminase activity was found to be normal in the $B_6$ deficient rats and to undergo the usual adaptive increase following the superimposition of $NH_4Cl$ acidosis.

It is concluded from these data that suppression of renal transaminase does not impair the renal production of ammonia, and that the level of renal transaminase activity is not important in renal production of ammonia either in normal or acidotic rats.

### Quantitative Measurements and Distribution of Alkaline Phosphatase in the Anatomic Units of the Human Nephron

By *Sjoerd L. Bonting, Victor E. Pollak, Robert C. Muehrcke and Robert M. Kark.* Departments of Medicine, Presbyterian Hospital, Cook County Hospital and Research and Educational Hospitals, Chicago, and Departments of Medicine and Biological Chemistry, University of Illinois College of Medicine. (Aided by a contract from the Surgeon General's Office, Department of the Army.)

The broad purposes of our investigations are to determine *quantitatively* the activity of enzymes and concentrations of various substances in the anatomic units of human kidney. This communication describes the analysis and distribution of renal alkaline phosphatase.

Animal tissue obtained immediately after death and human percutaneous renal biopsy samples were frozen rapidly in liquid nitrogen. The ultramicro technics of Lowry were adapted for renal tissue. Serial sections, cut at 16 $\mu$ in a cryostat (−20 C.), were lyophilized. Portions of single glomeruli, proximal or distal convoluted tubules, medullary ray, and vessel were dissected separately under a microscope from frozen-dried sections. Each microscopic specimen was weighed on a quartz-fiber "fish-pole" balance (sensitivity 0.4 m $\mu$g.; range 10–100 m $\mu$g.), and analyzed for alkaline phosphatase, the results being expressed in moles substrate split /Kg./hr. (MKH).

Alkaline phosphatase distribution was deter-

mined in man, monkey, dog, rabbit and rat. It differed markedly from one species to another. In all species highest activity was in the proximal tubules, but it varied from nephron to nephron (individual tubules of one species S.D. = 8.0 MKH; homogenates S.D. = 0.8 MKH). In normal human kidney higher activity was demonstrated in proximal (5.8 ± 1.1. MKH) than in distal tubules (2.9 ± 0.4 MKH). Glomerular activity was low (0.4 ± 0.16 MKH). In lupus nephritis glomerular activity was higher (0.7 ± 0.15 MKH), tubular activity lower (proximal 2.8 ± 0.8; distal 1.6 ± 0.1 MKH). In renal glycosuria values were comparable to lupus nephritis, but in hypophosphatasia they were extremely low (0.2 MKH).

Conclusions: (1) It was possible to dissect accurately the individual parts of the nephron and to make quantitative analyses thereon. (2) There were significant differences in enzyme activity in normal and abnormal tissues. (3) As there was no glycosuria in hypophosphatasia with minimal enzyme activity, and in lupus nephritis with activity comparable to renal glycosuria, alkaline phosphatase did not play a significant role in glucose reabsorption.

**Inhibition of Antidiuretic Hormone Activity As the Mechanism of Promazine-induced Diuresis**

By *W. Pierce Smith, Solomon Papper and Jack D. Rosenbaum.* Medical Service, Boston V. A. Hospital, and Departments of Medicine, Boston University School of Medicine and Tufts University School of Medicine, Boston.

An inhibitory effect on antidiuretic hormone (ADH) release has been suggested for promazine compounds on the basis of rat assays by Parrish and Levine. Diminished ADH activity in man may be presumed when urine flow and free-water clearance increase without concomitant rise in filtration rate and osmolal clearance. These parameters were studied in 12 subjects free of cardiovascular, endocrine or renal disease, who received, by intramuscular injection, 25 to 50 mg. of promazine hydrochloride (10 experiments) or chlorpromazine hydrochloride (2 experiments) at a time that they were on the descending limb of a water diuresis. In 11, urine osmolality declined significantly (by 34 to 366mOsmols) within the first 90 minutes after drug administration, accompanied by increase in urine flow and free-water clearance which averaged 2.7 and 2.6 ml./min. respectively. Endogenous creatinine clearances underwent no consistent alteration. Osmolal clearances rose slightly in 7 studies; the magnitude in the rise was not of the order which would be expected to lead to the observed increases in free-water clearance. Nevertheless an effect on ADH release could not be assumed with complete assurance. In 4 studies the increase in urine flow and free-water clearance together with the decline in urinary osmolality was associated with a decrease in osmolal clearance, thus strongly suggesting that the drugs inhibited ADH release.

**The Effect of Meperidine Hydrochloride upon the Renal Excretion of Water and Solute in Man**

By *Solomon Papper, Joseph L. Belsky, Kenneth H. Bleifer and W. Pierce Smith.* Medical Service, Boston V. A. Hospital, and Departments of Medicine, Boston University School of Medicine and Tufts University School of Medicine, Boston.

It is commonly stated that the diminished urine flow consequent to morphine or meperidine administration results from the stimulation of antidiuretic hormone release. Since we have found that morphine, in therapeutic doses, may result in decreased water diuresis without antidiuretic hormone release in normal subjects, the effect of meperidine was next investigated.

An oral water load of 20 ml./Kg. of body weight was established and maintained in 16 healthy adult recumbent subjects. At the height of the diuresis (11.9 to 22.5 ml./min.), 100 to 185 mg. of meperidine hydrochloride were administered subcutaneously or intramuscularly. Within approximately one hour, urine flow diminished in 15 of the 16 subjects by an average of 3.6 ml./min. (range 2.1 to 9.4). In the remaining subject antidiuresis was delayed until after the 2nd hour. An increase in urinary osmolality of minor magnitude (22 mOsm./L.) was observed in 5 subjects. This was quite transient in 3, and sustained in 2, both of whom suffered from unpleasant side effects of the drug (vomiting, vertigo). In the remaining 11 subjects urinary osmolality was unaltered. Endogenous creatinine clearance decreased by 10 to 32 ml./min. in 10 of the studies, rose by 15 ml./min. in 1, and was unaltered in 5. Total solute excretion decreased by 40 to 554 micro-Osmols/min. in 15 subjects and was unaltered in the other.

These observations indicate that the antidiuretic effect of meperidine, like that of morphine, may be independent of antidiuretic hormone release, and may be the result of a reduction in glomerular filtration rate and total solute excretion.

### Hyponatremia with Coma Following Chlorothiazide

By *Keith S. Henley, David H. P. Streeten and H. Marvin Pollard.* Department of Internal Medicine, University Hospital, Ann Arbor.

This study records the occurrence of severe hyponatremia in 4 patients treated with Chlorothiazide.

The patients responded to the drug with the anticipated diuresis and fall in body weight. In 2 of the patients, suffering from congestive heart failure and essential hypertension respectively, continued administration of Chlorothiazide (0.5 Gm. b.i.d. and q.i.d.) for 5 and 7 days on an 800 mg. sodium diet resulted in a fall in serum sodium concentration to 124 and 125 mEq./L. respectively, with weakness, dizziness, anorexia, vomiting and postural hypotension. A 3rd patient with parenchymatous liver disease became lethargic and lapsed into coma after 5 doses of the drug (0.5 Gm. per dose). At that time her serum total base concentration was 126 mEq./L. She improved rapidly upon intravenous administration of hypertonic saline.

The 4th patient, with cirrhosis and intractable ascites, tolerated the drug well in doses of 1.0 Gm. b.i.d. for several weeks, but remained in positive sodium balance on a 200 mg. sodium diet. Following bilateral adremalectomy, the ascites diminished progressively on maintenance therapy with hydrocortisone alone. To hasten the reduction of ascitic fluid, Chlorothiazide (1.0 Gm. b.i.d.) was administered for one day only, resulting in loss of 1.3 Kg. in weight, followed by incontinence and a clinical picture indistinguishable from deep hepatic coma. The serum sodium concentration was 111 mEq./L. The patient regained consciousness and was asymptomatic within an hour of the intravenous infusion of hypertonic saline. Subsequently, the ascites disappeared completely and symptoms of hyponatremia have not returned in the 6 weeks since Chlorothiazide treatment was stopped. These findings indicate that severe reductions of serum sodium concentration may occur as a result of Chlorothiazide therapy. Since the potency of the drug is considerably enhanced by the absence of adrenal tissue, Chlorothiazide should be used with particular care in patients with adrenal insufficiency.

### The Excretion of Acid in Renal Disease

By *Oliver Wrong and H. E. F. Davies.* Manchester, England.

Impaired ability to excrete acid complicates many forms of renal disease, yet it is not clear how this function can best be tested. Albright and others have studied the renal response to a metabolic acidosis induced over several days, but this procedure is too time-consuming for routine use and there is need for a shorter test.

In 10 normal subjects the ingestion of ammonium chloride 0.1 Gm./Kg., resulted in a mild acidosis with an average drop in plasma $CO_2$ of 4 mM/L. Urinary changes followed rapidly:—from 2 to 8 hours after ingestion was complete the urine pH was consistently under 5.3 and ammonium excretion had risen to 33–75 $\mu$Eq./min.

Over 50 patients have been studied by this procedure. An abnormally high minimum urine pH was regularly encountered in the classic syndrome of renal tubular acidosis (urine pH 5.7–7.0) and in severe potassium depletion pH 5.5–6.2). But patients with severe renal disease without evidence of tubular abnormality could usually excrete urine of pH 5.3 or under; many of these patients were clinically uremic and acidotic.

The rate of ammonium excretion correlated well with the glomerular filtration rate, and was invariably depressed in severe renal failure. Reduced ammonium excretion was found in 8 out of 9 patients with renal tubular acidosis, but does not appear to be a specific part of this syndrome, as glomerular filtration was correspondingly reduced.

We have studied 3 patients with what appears to be an incomplete form of the Butler-Albright syndrome of renal tubular acidosis. None of these patients has a systemic acidosis; all have generalised nephrocalcinosis without hypercalcuria and are unable to excrete urine of normal minimal pH. All 3 have relatively normal glomerular filtration rates and high ammonium excretion which prevents them from developing acidosis.

### Calcium and Magnesium Studies During ACTH Induced Diuresis in the Nephrotic Syndrome

By *Francis X. Fellers and Robert Schwartz.* Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston. (Aided by a grant from the National Heart Institute.)

Since hypomagnesemia was demonstrated previously to develop during diuresis in nephrosis, an investigation of calcium and magnesium metabolism in the course of the nephrotic state was made.

Complete 3-day balance studies over 39 and 33 days were obtained on 2 children with active disease, but normal creatinine clearances. ACTH at 150 mg./M²/day was given for 10 days and resulted in complete diuresis in each.

During the control periods, calcium and magnesium absorption was diminished in both patients. There was marked renal conservation of calcium, but to a lesser extent magnesium. In contrast, a 3rd patient with persistently low serum magnesium (1.4–1.6 mEq./L.) excreted no urinary magnesium.

In the prediuresis ACTH periods, there was further decrease in the apparent intestinal absorption of calcium and magnesium. Urinary calcium increased minimally from less than 0.1% to 0.4% of intake, while urinary magnesium decreased significantly. Balance data of nitrogen, phosphorus, potassium and magnesium suggested a loss of protoplasmic constituents.

With diuresis, there was marked increase in urinary loss of both calcium and magnesium such that 5 and 11% of calcium intake and 19 and 60% of magnesium intake was excreted. The urinary calcium was less than that contained in the lost edema fluid; however, the total magnesium loss approximated that estimated to be in edema fluid. This loss of magnesium, when serum magnesium was significantly diminished, suggests a renal defect in magnesium transport during diuresis.

The interrelations of calcium and magnesium metabolism during nephrosis and the steroid induced diuresis showed: (1) ACTH exaggerated the increased fecal loss of both calcium and magnesium, and (2) renal conservation was adequate until the diuretic phase when magnesium, but not calcium, excretion was excessive.

### Albumin Metabolism in Nephrotic Adults

By *Alan L. Kaitz.* Department of Pediatrics, Harvard Medical School, Children's Medical Center, and Nephritic Clinic, Beth Israel Hospital, Boston. (Aided by grants from the National Foundation for Infantile Paralysis and the National Institute of Arthritis and Metabolic Diseases, Public Health Service.)

An increased fractional rate of catabolism of albumin contributes significantly to albumin deficiency in some nephrotic children according to previous studies from this department. A study of nephrotic adults was carried out to determine whether an abnormality of albumin metabolism occurred in the adult as well as in the childhood type of nephrosis.

Each of 6 nephrotic adults was given an intravenous tracer dose of $I^{131}$-labeled human serum albumin and the $I^{131}$ activity in the plasma and urine was measured. Serum and urinary albumin was determined immunologically. The rates of urinary loss, catabolism, and synthesis of albumin were calculated from these data.

Three adult patients had *latent* nephrosis with little or no edema and minimal hypoalbuminemia while 3 had *overt* nephrosis with edema and moderate hypoalbuminemia. In the patients with *latent* nephrosis the fractional rate of catabolism was normal and the amount of albumin synthesized daily was sufficient to compensate for the urinary loss with little, if any, reduction in the total pool of albumin. By contrast, one of the 3 patients with *overt* nephrosis, a 30-year-old male, had a markedly increased fractional rate of catabolism contributing as much to the increased albumin turnover and deficiency as the massive urinary loss. In this patient the rate of albumin synthesis was increased. The fractional rate of catabolism of albumin was normal in the other 2 patients with *overt* nephrosis, women age 50 and 56, but impairment of albumin synthesis appears to have contributed to their albumin deficiency.

In overtly nephrotic adults, therefore, both increased catabolism and impaired synthesis of albumin, as well as albuminuria, may contribute to the reduction in size of the body pool of albumin.

### Tubular Changes in Acute Glomerulonephritis

By *A. E. Parrish, J. S. Howe and M. F. Watt.* Departments of Medicine and Pathology, George Washington University School of Medicine, and V. A. Hospital, Washington, D. C.

Tubular changes, although recognized, have not been emphasized in acute glomerulonephritis. The circulation of the kidney is such that with severe glomerular disease, especially when the lumina of the glomerular capillaries are diminished in size, it would be anticipated that tubular lesions would be present. Needle biopsy of the kidney has permitted serial observations in patients with this disease. In 24 such patients a correlation between the pathology seen in biopsy material, renal functions (inulin, urea clearance and PSP or TmPAH), and the clinical course has been attempted. In the patients studied, all have shown tubular pathology at some time in the

course of their disease. Early (within the first week) pyknosis and vacuolization were present in tubular cells. In 2 instances this was severe and accompanied by a clinical picture of "lower nephron nephrosis." Renal functions showed marked depression of TmPAH and a low specific gravity. Later in the course of the disease tubular dilatation became prominent and was seen as long as 3 years after the onset of the disease. Tubular dilatation was not associated with decreased renal function (TmPAH) unless other abnormalities were also present. Persistent interstitial tissue abnormalities such as edema, cellular infiltration, and fibrosis seemed to be the changes associated with poor prognosis.

### The Hypervolemic Syndrome Associated with Acute Glomerulonephritis

By *Seymour Eisenberg*. Medical Service, V. A. Hospital, and Department of Medicine, University of Texas Southwestern Medical School, Dallas.

Recent interest in atypical congestive syndromes prompted this investigation of the blood volume alterations in persons with acute glomerulonephritis. Eight subjects with serologically proven acute, poststreptococcal glomerulonephritis were studied. Edema, venous hypertension, and cardiac enlargement were present in every instance. Radiochromium-labeled erythrocytes were employed as a reference, from which red cell mass was measured and total blood and plasma volumes were calculated. Following relief of the congestive phenomena, the measurements were repeated.

The results indicate that the blood volume is greatly expanded (29%) during the edematous phase of acute glomerulonephritis as a result of a selective increase (38%) in plasma volume; these alterations are associated with a significant lowering (6 mm.) of the hematocrit. Following spontaneous diuresis, the blood and plasma volumes returned to normal. In a previous study, patients with congestive heart failure were found to have a 22% increase in blood volume, a 25% increase in red cell mass, and a 17% increase in plasma volume; the pattern of blood volume expansion, therefore, contrasted sharply with that of the subjects with nephritis. In 3 persons with the nephrotic syndrome the blood volume was unaltered.

It is suggested that expansion of the intravascular volume plays a key role in the pathogenesis of this and other congestive syndromes of the type described by Eichna. This concept is supported by previously reported hemodynamic alterations in persons with acute nephritis. The selective increase in plasma volume undoubtedly reflects the primary renal retention of salt and water in this disorder. The apparent anemia of the subjects studied was a dilution phenomenon, as the hematocrit values increased to normal following recovery without a significant change in red cell mass.

### Studies in Experimental Pyelonephritis: Simultaneous and Serial Investigation of a Pyelonephritic and Intact Kidney in the Same Animal

By *Neal S. Bricker, Richard R. Dewey and Herbert Lubowitz*. Departments of Medicine and Preventive Medicine, Washington University School of Medicine, St. Louis.

While pyelonephritis is an extremely common disease, it has been studied extensively only during recent years, and information regarding pathogenesis, pathologic physiology and natural history is limited.

In the present studies, unilateral pyelonephritis has been induced in dogs previously subjected to a surgical procedure (bladder-splitting) which permits simultaneous and serial collection of urine from the separate kidneys. The experimental model so produced lends itself to examination of bacteriologic, functional, and histologic parameters of a pyelonephritic kidney, allowing comparison with concurrent observations from the intact organ.

The technic for inducing unilateral pyelonephritis consists of exposing one kidney through a flank incision, clamping the ureter for 30 minutes, massaging the kidney to render it hyperemic and puncturing the renal parenchyma to a depth of approximately 6 mm. in 100 to 150 sites. Prior to removal of the ureteral clamp, one ml. of a 4-hour culture of pathogenic bacteria is injected intravenously.

Within 3 days, bacteria may be cultured both from the parenchyma and urine of the experimental kidney. Positive urine and biopsy cultures persist indefiintely and re-injection of bacteria increases the rate of bacteriuria. Three days after the experimental procedure, the involved kidney is enlarged and hyperemic. Within 3 weeks, an absolute decrease in renal mass generally occurs. Histologically, experimental kidneys show classic changes of pyelonephritis. Control kidneys show no consistent abnormalities.

Serial renal function studies demonstrate a

decrease in GFR and RPF in the experimental kidney. Values for filtration fractions, however, do not deviate appreciably from those of the control organ. Moreover, per unit of glomerular filtrate, concentrating capacity ($T^{c}_{H_2O}$) and diluting capacity ($C_{H_2O}$) remain comparable to the intact kidney. These observations fail to demonstrate a functional pattern distinguishing pyelonephritis from other forms of progressive renal disease and suggest that the persisting nephrons in pyelonephritic kidneys retain normal functional characteristics.

### A Search for Unsuspected Pyelonephritis among Patients with Hypertension

By *Hans G. Grieble, Louis C. Johnston and George Gee Jackson.*

Many patients with hypertension have been reported to have clinically unsuspected pyelonephritis. In our studies 87 ambulatory patients with hypertension without clinically apparent pyelonephritis have been examined at monthly intervals over 2 to 20 months for the presence of urinary infection. One-third of the patients had uremia or grade 2 to 4 hypertension. Nine have died after 4 to 15 months of study; 7 had autopsies and 1 had a kidney biopsy. More than 500 quantitative bacteriologic cultures and pale-cell preparations of the urine sediment have been performed.

Unsuspected urinary infections were found in 8 patients; 6 had no prior history or symptoms of genitourinary infection. In one year 4.5% of the patients were observed to develop spontaneous infection. Twenty-two of 78 patients with a history of urinary infection and 4 of 56 patients without prior history or present infection had objective evidence of previous pyelonephritis. Altogether 20, or 23%, had active urinary infection, or previous pyelonephritis.

A single positive bacterial culture with $10^5$ or more bacteria/ml. of urine was 95% indicative of persistent infection. Catheter specimens from uninfected patients had growth to that extent in only 1%. A single voided urine specimen from females containing $10^5$ or more organisms per ml. indicated active infection in 80%. Among uninfected females a false positive culture occurred in less than 3% and was not accompanied in any instance by a positive pale-cell preparation of the sediment. The use of both quantitative cultures and pale-cell preparations of the urine sediment detected unsuspected infections in 10% of patients. A single urine specimen positive by both criteria was 96% accurate in the diagnosis of active infection.

### Structure and Function in Diabetic Nephropathy: The Importance of Diffuse Glomerulosclerosis

By *Derek D. Gellman, Conrad L. Pirani, John F. Soothill, Robert C. Muehrcke, William Maduros and Robert M. Kark.* Departments of Medicine, Presbyterian–St. Luke's Hospital, Cook County Hospital and the Research and Educational Hospitals, Chicago; and Departments of Medicine and Pathology, University of Illinois College of Medicine. (Aided by grants from the United States Public Health Service and Eli Lilly and Company.)

In order to assess the functional significance of kidney involvement in diabetes mellitus, 53 diabetic patients, with and without clinical evidence of renal disease, were studied by renal biopsy. The histologic lesions were graded according to severity and correlated with the clinical findings and laboratory tests. Some patients were studied serially, 63 biopsies being made.

The results differed from those of most other workers who have based their studies on autopsy material. It was shown that, from the functional point of view, diffuse diabetic glomerulosclerosis is a more important lesion than the better known Kimmelstiel-Wilson nodule.

Nodular glomeruiosclerosis (Kimmelstiel–Wilson) was found in 53%, including 9 of 16 patients aged 30 or less. Its occurrence was significantly correlated with the duration of diabetes, but not with the age at which diabetes became manifest nor with age at the time of biopsy.

Diffuse diabetic glomerulosclerosis (Bell) was found in 77%, including 14 of the 16 patients aged 30 or less. Our studies indicated that this is a specific lesion which can be clearly differentiated from membranous glomerulonephritis and arteriolar nephrosclerosis. Its incidence increased with increasing duration of diabetes; it was also commoner in those whose disease started early in life.

The complete nephrotic syndrome—not due to chance association—was present in 14 patients. The mean age at which these patients developed diabetes was 13 years less than that of the remainder, a statistically significant difference. The mean duration of disease was 4 years longer; this difference was not significant.

The severity of diffuse glomerulosclerosis correlated better than did nodular glomerulosclerosis with all tests of renal function—e.g.

urinalysis, proteinuria, serum urea and creatinine, urea and creatinine clearances; with the presence of oedema and other features of the nephrotic syndrome; and with the height of the diastolic blood pressure.

### Reversible Renal Failure in Rats: An Experimental Tool

By *Paul E. Teschan and Arthur D. Mason, Jr.* United States Army Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.

Etiologic settings and clinical manifestations in groups of patients with acute renal failure often vary too widely to evaluate conclusively the effect of treatment apart from the natural history of the syndrome or to study the pathogenesis of the renal lesion itself. This presentation describes the principal characteristics of a laboratory animal preparation by which acute reversible renal failure may be so studied. The use of rats has the additional virtue of providing inexpensive replication in statistically oriented experimental designs by which treatment effects can be discerned with certainty apart from the wide biologic variation between individuals with acute renal failure.

Sprague–Dawley rats (150–200 Gm.) are fed horsemeat, oats and water ad libitum for 4 days, oats alone for an additional 24 hours, and then receive an intravenous injection of 0.5 Gm./Kg. of a 15 Gm.% ferricyanide-converted methemoglobin solution together with 14.3 mg./Kg. of sodium ferrocyanide. Horsemeat, oats, and water are then continued ad libitum for 4 days after which the standard rat pellet diet is resumed.

Acute renal insufficiency occurs in approximately 80% of the animals so treated as measured by an elevated plasma urea nitrogen averaging 176 mg.% 48 hours following injection. Oliguria, diuresis, marked hyperkalemia, reduced plasma sodium and bicarbonate concentrations, a notable clinical illness in ⅓ of individuals, and an approximate mortality of 20% are regularly found. Study of renal morphology following injection reveals normal glomeruli and a regular sequence of tubular changes: casts, interstitial edema, widespread tubular necrosis, active tubular regeneration, interstitial cell infiltrate, and restoration of normal architecture with persistent, infrequent foci of necrosis and scarring.

Analogy to the human disease is demonstrated by every criterion so far examined, the chief difference being a more rapid cycle of chemical and histologic changes in the rat.

### The Effect of Chronic Water Loading on Solute and Water Excretion in Uremia

By *Warren R. Guild, John V. Young and John P. Merrill.* Medical Service, Peter Bent Brigham Hospital, and Department of Medicine, Harvard Medical School, Boston.

Previous studies have demonstrated that acute water loads were ineffective in increasing total solute excretion in patients with chronic renal failure except when they result in hypotonic expansion of extracellular fluid. Such water loads are poorly tolerated. In the following studies the effect of water loading by small successive daily increments was evaluated. Three patients were studied in detail. Patients were placed on a constant diet, but were allowed fluid ad lib for a 4–5 day control period. The average fluid intake was chosen as a baseline and daily water intake was then increased by successive 500 cc. increments per day during the experimental period of 7–10 days. Peak water intakes averaged 230% of control period. Urine volumes changed in corresponding fashion. At the peak of the increase in urine volume, however, the total 24-hour solute excretion was insignificantly changed (90–105% of control). A marked drop in urine osmolarity explained this failure of total solute increase. No significant change in body weight, hematocrit or total sodium excretion accompanied the increase in urine volume. The results indicate that while patients with chronic renal failure can significantly increase urine volume and decrease urine concentration, with water loading this increased volume is unaccompanied by concomitant increase in total solute or sodium excretion. Unlike experiments with acute water loading, gradual increments of fluid are excreted and do not result in hypotonic expansion of extracellular fluid and increased filtration rate which may in some of the acute studies account for increase in solute excretion occasionally observed, as well as the symptoms of water intoxication.

### Dialysance of Tritium with the Twin Coil Artificial Kidney

By *John E. Kiley, Robert L. Barenberg and William H. Conklin.* Department of Medicine, Albany Hospital, and Albany Medical College, Albany, New York.

The use of hemodialysis to remove tritium

from the body in cases of accidental overdosage has been suggested. The dialysance of tritium by the disposable twin coil kidney was determined during 3 in vitro dialyses of 4 to 5 L. of blood containing approximately 4 μc. of tritium per ml. Tritium was assayed by liquid phosphor scintillation-counting. Pressure, flow, and dialysance of urea were also determined. Typical values were as follows: pressure, 270 mm. Hg; flow, 260 ml./min.; tritium dialysance, 185 ml./min.; urea dialysance, 121 ml./min. The high dialysance of tritium indicates that hemodialysis would effectively remove tritium from the body and suggests that generally over 50% of the water in the blood is exchanged in each passage through the twin coil artificial kidney.

### Gastrodialysis in the Treatment of Acute Renal Insufficiency

By *Belding H. Scribner, William R. Koreski, Thomas A. Marr and James M. Burnell.* University of Washington, School of Medicine, Seattle.

Previous attempts to use gastric lavage or gastrodialysis in the treatment of patients have failed because of uncontrollable electrolyte transfers and loss of large amounts of dialysis fluid into the patients. These problems have largely been solved as a result of the introduction by Schloerb of the cellophane bag technic of gastrodialysis.

An apparatus has been devised that cycles fluid at body temperature through the cellophane bag. The apparatus consists of a timing clock that rotates a cam which operates switches controlling solenoid values, and electric pumps that cycle the fluid in and out of the bag. A safety switch stops the cycling process if excessive fluid losses should occur, thus permitting operation of the apparatus without constant supervision.

Experience in the management of 10 patients with acute renal insufficiency suggests that gastrodialysis is entirely effective in the treatment of acidosis and a useful adjunct in potassium, water and nitrogen removal and glucose administration.

Preliminary experience shows that the most rapid transfers affect the acid-base status of the patient. Chloride-bicarbonate transfers have been of large magnitude. Hydrogen-sodium transfers have been smaller. The combined transfers have resulted in the rapid production of metabolic alkalosis. Potassium removal has varied from 6 to 50 mEq./24 hrs. The average has been 25 mEq./24 hrs. With isotonic solutions no transfer of water has occurred, whereas, hypertonic solutions of 20–40% glucose have removed up to one liter per 24 hours. Nitrogen removal has ranged from 0.5 to 4.1 Gm./24 hrs. The average has been 2.4 Gm./24 hrs. Glucose transfers vary with the concentration of glucose. From 100 to 800 Gm. have been given by this means.

Results of these studies indicate that gastrodialysis is a useful adjunct in the management of the electrolyte disorders of acute renal insufficiency. It will delay and in some cases prevent the onset of the uremic syndrome.

### Water and Solute Movements during Intermittent Peritoneal Dialysis in Human Subjects

By *Morton H. Maxwell, Charles R. Kleeman and Robert E. Rockney.* Department of Medicine, University of California Medical Center, and V. A. Hospital, Los Angeles. (Aided by grants from the Arie Crown Foundation and the Viola and Alfred Hart Foundation.)

Fluid and solute movements during intermittent peritoneal dialysis were studied in 44 experiments on 36 subjects with acute and chronic renal failure, intractable edema or hypercalcemia. A slightly hypertonic idealized "extracellular" electrolyte solution, with or without K, was administered rapidly in the midline through a rigid, curved, multi-eyed, polyvinyl catheter and subsequently drained by gravity in a closed system.

The peritoneum fulfilled criteria for an inert, semipermeable membrane. Equilibration between plasma and dialysis solution for K, Ca, $PO_4$, creatinine and urea followed a symptotic curve, approaching 75% in 1 to 1½ hours. Equilibration rates for these substances did not differ significantly when the total volume of administered solution was 2,3 or 4 liters, or when its osmolality was increased with 50% glucose. Average serum osmolality for moderately uremic patients was 328 mOsm./L. (normal: 287). One hour after infusing 2 L. of dialysis solutions with 2% glucose (387 mOsm./L.), 7% (630 mOsm./L.), and 10% (850 mOsm./L.), negative fluid balances averaged 225, 800, and 1090 cc., respectively. Up to 20 L. of fluid were removed in patients with intractable anasarca. Intermittent dialysis was usually continued for 24 hours. Total amounts of solutes removed in 12 hours averaged: K, 71 mEq.; BUN, 20.4 Gm.; creatinine, 2.3 Gm.; $PO_4$, 0.9 Gm. Average urea clearance was 24.6 and creatinine clearance 24.2 cc./min. When

hyperkalemia was present initially, serum K was decreased 1.4 to 4.2 mEq./L., in some cases with a decrease of over 1 mEq./L. in the first 2 hours of dialysis. In one patient with hypercalcemia and oliguria, 17 hours of dialysis reduced serum Ca from 25.6 to 12.2 mg.%; total Ca removed was 750 mg., of which only 250 mg. could be accounted for in the extracellular fluid.

It is concluded that intermittent peritoneal dialysis is an effective means of rapidly and selectively removing fluid or solutes from the extra- and intracellular compartments.

### The Excretion of Urea Nitrogen in Thoracic Duct Fluid in Man

By *Philip D. Cronemiller, Ralph L. Byron, Jr. and Howard R. Bierman.* Duarte, California.

The thoracic duct fluid of man contains a concentration of urea nitrogen equal to that of the blood. Thoracic duct fluid is a cell-poor liquid containing about half the protein content of plasma. These studies were an attempt to lower the blood urea nitrogen level in renal failure states by drainage of thoracic duct fluid.

The procedure was carried out under local anesthesia. The duct was identified near its entrance into the jugular-subclavian junction and a small polyethylene catheter passed into it in a retrograde manner. The fluid was collected by free gravity drainage and replaced volume for volume with intravenous administration of plasma and whole blood. No problem in electrolyte equilibrium was encountered.

Five patients with far advanced neoplastic disease and renal failure were studied. Four patients had bilateral ureteral obstruction and one had a severe hepatic failure with renal shutdown. One study was a technical failure because of an inadequate flow of thoracic duct fluid. In the 4 remaining cases, thoracic duct flow ranged from 1200 to 10,000 cc. (800–2,000 cc./24 hrs.). The blood urea nitrogen ranged from 80 to 150 mg./100 cc. of blood (average 123) at the start of the procedure, and was decreased to 34 to 90% of the predrainage uremic level. Clinical improvement was noted in 3 of these 4 patients.

Thoracic duct drainage may be of therapeutic significance in the temporary control of conditions of impaired renal function which is reversible.

### The Effect of Spinal Cord Injury on Renal Function

By *J. H. Magee, S. A. Pennisi, A. M. Unger, L. W. Holladay and R. C. Bunts.* Spinal Cord Injury and Urology Services, V. A. Hospital, and Department of Medicine, Medical College of Virginia, Richmond.

Renal function was measured in 19 patients, 34–338 days after traumatic spinal cord injury. Clearance of inulin ($C_{IN}$) and of sodium para-aminohippurate ($C_{PAH}$) were used to measure glomerular filtration rate and effective renal plasma flow. Subjects were studied soon after transfer, in most cases from military or other V. A. hospitals. Weight at the time of study averaged 21.25 (17.75 ± 22.5) pounds less than reported pre-injury weights. Calculated surface area averaged 0.13 square meters ($M^2$) less than that calculated from pre-injury weights. All patients were free of the severest manifestations of catabolism such as decubitus ulcers, and all had received testosterone propionate for varying periods before study.

Average $C_{IN}$ in 21 measurements on 19 patients was 122.8 cc./min./1.73 $M^2$ (120 ± 29); average $C_{PAH}$ in 20 measurements on 19 patients was 643 cc./min./1.73 $M^2$ (610 ± 154.5); average filtration fraction was 0.189 (0.20 ± 0.05). $C_{IN}$ was less than the 99 cc./min. (uncorrected), proposed by Morales et al. as an arbitrary lowest normal figure in their patients, in only 4 of this group (91, 91, 94, and 96 ml./min.). Cystography has revealed vesicoureteral reflux in 2 of these patients.

These studies within the first year after spinal cord injury are felt to constitute evidence against significant renal functional alteration solely as a consequence of immobilization, catabolism, altered body composition and surface area, and sympathetic denervation. They are inconclusive as to, but not inconsistent with, the prevailing impression that renal dysfunction following spinal cord injury is a consequence of infection.

### A Familial Form of Hyperphosphaturia, Resembling Achondroplasia, with Dwarfing, Multiple Exostoses, and Bowing

By *Glenn E. Mortimore, Felix O. Kolb, Francis S. Smyth and Howard L. Steinbach.* Departments of Medicine, Pediatrics, Radiology and the Metabolic Unit, University of California School of Medicine, and V. A. Hospital, San Francisco.

Several members of a family have been studied who showed a striking picture of dwarfism, bowing of the legs, painful limitation of joint movement, peculiar "turret head," and premature loss of teeth. The biochemical abnormality observed was a marked hypophosphatemia in the presence of hyperphosphaturia, and elevation of the serum alkaline phosphatase. The absence of hypercalcemia or hypercalciuria ruled out primary hyperparathyroidism. The bone structure, in contrast to ordinary rickets or osteomalacia, showed increased density, with short, broad tubular bones, thick cortices and exostoses about joints. In addition, a child showed evidence of rickets at the metaphyseal margins. The skull was deformed, resembling craniostenosis. Biopsies obtained before and after treatment showed evidence of osteomalacia of mild degree with subsequent healing. Phosphate clearances showed, in the adult members, a definite renal phosphate loss, with low values for tubular reabsorption of phosphorus (% T.R.P.). In the child, however, this phosphaturia did not become apparent until after initiation of treatment with vitamin D. It was further noted that while healing of the bones, with intensive vitamin D therapy, was accompanied by a fall in serum alkaline phosphatase toward normal, the serum phosphorus level remained low and the renal phosphate loss remained virtually unchanged. Administration of parathyroid hormone to one of the subjects markedly decreased the tubular reabsorption of phosphate. These findings suggest (1) that the phosphaturia is primarily a tubular defect, which is not due to hyperparathyroidism, and (2) that healing of the bone lesions occurs with large doses of vitamin D by a mechanism other than decreasing the renal phosphaturia. One child, age 5, free of gross physical signs, but discovered to be involved by a survey of her family, has shown healing of rickets after treatment for 2 years. The permanent deformities, resembling achondroplastic dwarfism, and loss of teeth, present in the untreated adults, may thus be prevented.

## LIVER

### The Use of the Glutamic Oxaloacetic Transaminase Test For Detection of Hepatitis Among Blood Donors

By *Paul Ruegsegger, Nils Bang, Allyn B. Ley and John S. LaDue.* Sloan-Kettering Division of Cornell University Medical College, New York.

The serum glutamic oxaloacetic transaminase activity of 13,266 donor bloods was measured in an attempt to evaluate this test as a method of detecting the presence of hepatitis carriers. The SGO-T activity was above 41 to 50 units in 8.54%, to 100 units in 12.55% and above 100 units in 2.14%. The peak incidence of elevation of SGO-T activity was in December at a time when the reported cases of viral hepatitis was high in the New York City area. The sex rate of increased SGO-T activity was 3:1 in favor of the male donors.

Of 4,240 patients who received one or more blood transfusions during the period of study, 1,370 were available for detailed follow-up. Nine patients or 1.5% of the 603 recipients who received only blood with normal transaminase activity developed hepatitis, while 30, or 3.9% of the 768 recipients of one or more bloods with an abnormal transaminase activity developed hepatitis. In this latter group of 768 recipients of abnormal blood, 144 received one or more bloods with highly abnormal values of more than 100 units, and of these, 8 patients or 5.5% developed hepatitis. There is a statistically significant difference between the incidence of hepatitis in the group that received only normal bloods and the group that received one or more abnormal bloods. There was no consistent relationship between donor bloods with increased SGO-T activity and their SGP-T activity, thymol turbidity and bilirubin levels.

### Glutamic Oxaloacetic Transaminase Activity in Acute and Chronic Alcoholism

By *Nils Bang, Kurt Iversen, Tove Jagt and Sten Madsen.* Kommune Hospitalet, Second and Third Division of Medicine, Copenhagen, Denmark.

The purpose of the present investigation has been to follow the serum glutamic oxaloacetic transaminase (SGO-T) activity during and after heavy alcohol ingestion.

The SGO-T activity, the icteric index (II),

the thymol extinction (TE), and the serum proteins measured by paper electrophoresis were determined repeatedly for the first 3 to 11 days following excessive alcohol ingestion in 35 chronic alcoholics, and the results were correlated with the blood alcohol levels on admission to the hospital. The same blood tests were performed at comparable time intervals in an additional 19 chronic alcoholics who had no access to alcohol and in 12 healthy, nonalcoholic individuals after the administration of large amounts of alcohol.

The SGO-T activity was elevated in 27 of the 35 patients with chronic alcoholism following acute alcohol intoxication, while the TE test, the II, and the serum proteins remained within normal limits. Elevated SGO-T activities were encountered as early as 2 hours after the intake of alcohol and abnormal values persisted as long as 11 days after the alcohol ingestion. A rough correlation was found between the blood alcohol concentrations and the maximum SGO-T activities.

No change in SGO-T activity, II, TE or serum proteins was found in 12 healthy volunteers after ingestion of 250 cc. of aquavit although comparable blood alcohol levels developed. In the 19 chronic alcoholics denied access to alcohol for 1 to 18 months all tests on repeated observations remained within normal limits. All of these patients were on a nutritious diet. One of the patients was given an "alcohol tolerance test": 200 and 300 cc. of aquavit was administered 2 and 5 weeks after hospitalization. On both occasions significant increase in SGO-T activity without change in other chemistries was found. These findings suggest that the cells of the liver of the chronic alcoholics are damaged by repeated ingestion of alcohol.

### Radioactive Fat and Fatty Acid Studies in Patients with Jaundice

By *Donald Berkowitz and David Sklaroff*. Departments of Medicine and Radiology, Albert Einstein Medical Center, Northern Division, Philadelphia.

Recent experiences with radioactive triolein test meals have demonstrated the usefulness of this technic in the study of fat absorption in various disease states. After the oral ingestion of a measured amount of this material, characteristic blood absorptive patterns are obtained. A flat curve is indicative of malabsorption and is seen in sprue and pancreatic insufficiency.

We have studied 40 patients with jaundice with this procedure. In 20, the etiology was an extrahepatic block confirmed by operation or autopsy. In the other 20, the clinical course, plus liver biopsy material, indicated a parenchymal type of jaundice. In those with obstructive jaundice, the average blood isotope level was markedly depressed with a narrow range in the entire group. In the nonobstructed group, this value was much higher, although lower than normal.

In a number of patients who demonstrated flat triolein responses, a test meal of tagged oleic acid was also given. A normal absorption curve in this instance confirmed the existence of an obstructive process and indicates that the presence of bile is not necessary for adequate absorption of fatty acids.

These radioactive fat tests correlate well with the clinical and laboratory findings, and were actually of diagnostic significance in several instances where the etiology of the jaundice was in doubt.

### Diurnal Variation of Urinary Coproporphyrin Excretion

By *John T. Galambos and Richard G. Cornell*. Emory University School of Medicine, Department of Medicine, Section of Gastroenterology; Communicable Disease Center, USPHS, DHEW; and Grady Memorial Hospital, Atlanta.

The evaluation of the 24-hour urinary coproporphyrin (UCP) excretion is one of the most sensitive indicators of hepatocellular injury and was shown to be one of the four most discriminating liver function tests in hepatitis and cirrhosis. As in the case of most quantitative tests, the 24-hour UCP excretion overlaps in normals and in patients with liver injury. A hypothesis is proposed that in a case of normal UCP excretion the diurnal variation becomes more pronounced before there is a significant elevation of the 24-hour UCP excretion. If this is correct, then a measure of the variability of the UCP excretion rate is a more discriminating and sensitive test as compared to the measurement of the total 24-hour excretion.

The UCP was measured by the ethylacetate extraction method of Schwartz et al. Consecutive 12-hour urines were analyzed in 9 healthy individuals for 10 to 20 periods and in 8 ill patients for 7 to 18 periods. Consecutive 4-hour urines were analyzed in 9 healthy individuals for 4 to 25 periods and in 10 ill patients for 6 to 12

periods. The 24-hour UCP excretion was also measured in these individuals.

The coefficient of variation (CV) for any individual equals 100 times the ratio of the standard deviation of the determinations for that individual to the mean of those determinations ($CV = \frac{\sigma}{\bar{x}} 100$). The CV based on 12-hour UCP excretions for the healthy group ranged from 12 to 39, and for the ill patients from 18 to 55. For 4-hour UCP determinations, the CV ranges from 8 to 32 for the healthy group, and from 39 to 74 for the ill group.

On the basis of 24-hour total UCP excretion, 2 healthy persons, 2 patients in the recovery phase of acute liver injury and one patient with cirrhosis would have been misclassified. However, on the basis of the CV for 4-hour UCP excretions, correct classification was possible.

The variation of the UCP excretion is a more sensitive index of normal or disturbed porphyrin metabolism than the 24-hour total, if the UCP excretion is not unusually low. On the basis of this preliminary study it is suggested that a normal CV of 4-hour UCP excretions is 30 or less; and the CV is abnormal if it is 40 or more.

### Effects of pH and Blood Gases Upon Ammonia Concentration

By *Curtis J. Fisher, Robert Eich and William W. Faloon.* Department of Medicine, State University of New York, Upstate Medical Center, Syracuse.

There has been conflicting evidence regarding the effect of varying oxygen concentrations upon blood ammonia. Furthermore, alterations in pH have been reported to affect the blood ammonia concentration.

Arterial oxygen concentration, pH, $CO_2$, and arterial blood ammonia have been determined in 6 cirrhotic patients who were exposed to varying concentrations of oxygen and nitrogen.

Successive determinations were performed (a) while breathing room air, (b) after exposure to 12% oxygen for 10 minutes and (c) after exposure to 100% oxygen for 10 minutes.

No correlation between changes in oxygen concentration and blood ammonia was noted. Only small changes in pH were produced and no correlation with changes in ammonia concentrations was observed.

Upon exposure to an atmosphere containing 12% oxygen, the $CO_2$ content of the arterial blood decreased in 2 patients and the ammonia concentration rose. A small increase in the arterial $CO_2$ occurred in the other 4 patients and was accompanied by a decrease in the ammonia concentration.

After breathing 100% oxygen, further changes in $CO_2$ and ammonia concentrations occurred. In 4 of the 6 patients the $CO_2$ increased and the ammonia decreased. In 2 patients the $CO_2$ decreased, the ammonia concentration declined in one and remained unchanged in the other.

The data suggest that arterial blood ammonia concentration may in part be related to $CO_2$ content, but is not related to changes in oxygen concentration and pH as produced in this study.

### Effect of Reduced Ammoniagenesis and of Ammonium Administration Upon Nitrogen Metabolism in Hepatic Cirrhosis

By *William W. Faloon, Curtis J. Fisher and Kathleen C. Duggan.* Department of Medicine, Upstate Medical Center State University of New York, Syracuse.

Previous studies have demonstrated that in cirrhotic patients receiving neomycin blood $NH_3$ falls, and when adequate diets are fed, nitrogen balance becomes positive or is unchanged despite increased fecal nitrogen loss. These observations have led to studies of the effect of ammonia upon nitrogen balance.

In 3 cirrhotic patients with ascites who received diets constant in nitrogen (0.19 Gm./Kg. of dry weight) and calories (26 to 28/Kg.), nitrogen balance was determined during successive 6-day periods of: (1) control, (2) neomycin 12 Gm./day orally, (3) neomycin plus ammonium citrate and chloride (2 Gm. $N_2$/day), (4) neomycin alone, (5) control. Venous blood ammonia concentrations were determined in all 3 patients and postprandial alpha-amino nitrogen and urea in 2. Two patients had elevated blood ammonia levels during control periods and the 3rd had normal levels.

Reduction in intestinal ammonia production by neomycin had no consistent effect upon nitrogen balance. During the periods of neomycin alone, blood ammonia fell significantly in the 2 patients with elevated levels; a slight fall occurred in the other patient. Administration of ammonium salts increased the blood ammonia in all patients to approximately twice the values seen during neomycin alone, but failed to pro-

duce levels as high as those observed during control periods. Nitrogen balance became positive during the ammonium salt administration in one patient, but was unchanged in the others. In the 2 patients studied, blood urea and alpha amino nitrogen fell slightly during neomycin administration and returned toward control values during ammonium administration and in the final control period.

The data indicate these conclusions: (a) the previously observed enhancement of nitrogen anabolism during neomycin is unlikely to be due to reduction in ammonia formation alone, (b) ammonium per se in the amounts used is not deleterious to nitrogen metabolism, (c) intestinal ammonia formation in the untreated cirrhotic is probably in excess of 2 Gm. of $NH_3$-$N_2$ daily.

### The Isolation and Measurement of Corticosteroid Glucuronides in the Plasma of Patients with Laennec's Cirrhosis

By *George L. Cohn and Philip K. Bondy.* Department of Internal Medicine, Yale University School of Medicine, New Haven. (Aided by grants from U.S. Public Health Service and James Hudson Brown Foundation.)

It has been suggested that the rate limiting step in the endogenous metabolism of cortisol is the reduction of the △-4-3 ketone to tetrahydro derivatives by the liver. Conjugation of the tetrahydro metabolites with glucuronic acid is then believed to follow so rapidly that no unconjugated tetrahydro derivatives reach the plasma or urine. The validity of this hypothesis has been investigated in 6 patients with advanced Laennec's cirrhosis proven by liver function tests and biopsy.

Tetrahydrocortisol and tetrahydrocortisone glucuronides were extracted from 20 cc. of plasma with absolute ethanol followed by *n*-butanol saturated with water. After washing with hexane, the extract was submitted to paper electrophoresis which separated nonionizable unconjugated steroids (e.g. cortisol) from glucuronides and sulfates. The glucuronide portion was fractionated by paper chromatography. Tetrahydrocortisol and tetrahydrocortisone glucuronides were measured by the carbazole reaction, using a Beckman DU Spectrophotometer ($\lambda$ 530 m$\mu$.)

Fifteen individuals without hepatic or endocrine disease, aged 19 to 55 years, had plasma concentrations ranging from 5.5 to 16.50 (mean ± standard deviation — 10.3 ± 3.51 $\mu$g./100 cc.) of tetrahydrocortisone glucuronide and 4.75 to 14.50 (8.1 ± 2.49 $\mu$g./100 cc.) of tetrahydrocortisol glucuronide. Five patients with Laennec's cirrhosis had less than 1.0 $\mu$g./100 cc. of plasma of tetrahydrocortisone and tetrahydrocortisol glucuronides. One patient with advanced healing cirrhosis, demonstrated on biopsy, had glucuronide levels in the low normal range. Although the plasma of cirrhotic patients contained no corticosteroid glucuronides, it did contain tetrahydrocortisone and tetrahydrocortisol.

These findings would seem to support the theory that the rate-limiting step in the metabolism of endogenous cortisol in the patient with advanced Laennec's cirrhosis is the conjugation of the 3 hydroxyl with glucuronic acid to form the glucuronide, rather than the reduction of the △-4-3 ketone to form the tetrahydro derivative.

### The Effects of an Aldosterone Antagonist in Decompensated Liver Disease

By *Robert S. Morrison and Thomas C. Chalmers.* Lemuel Shattuck Hospital and Harvard Medical School, Boston.

An increased urinary excretion of aldosterone has been found in many patients with cirrhosis of the liver with ascites. The renal effect of this hormone in producing a retention of sodium has been considered a link in the mechanism of ascites and edema. New steroids have been synthesized which appear to antagonize the action of aldosterone on the kidney. The effects of administration of one of these, (3-oxy-17$\beta$-hydroxy-19-nor-4-androsten-17$\alpha$-yl) propionic acid $\gamma$-lactone (SC 8109), was studied in 6 patients with cirrhosis of the liver.

Each patient was given a 200 mg. sodium diet; and daily fluid intake, urinary volume, and body weight were recorded. Serial determinations were made of serum and urinary concentrations of Na, K, Cl, $CO_2$, and creatinine. A microcrystalline suspension of the steroid was administered intramuscularly in divided doses totalling 25 to 200 mg./day. When the drug was given to 5 patients with definite ascites who had pretreatment levels of urinary sodium excretion below 1 mEq. daily, there was an increased natruresis to levels between 10 and 20 mEq. daily. This effect was observed either on the day of administration of the antagonist or the day following or both. The one patient with equivocal ascites had no response. Sodium excretion in the range of 37 to 75 mEq./day was observed in 3 patients following the administration of the antagonist and mercuhydrin on the same day;

whereas the same patients had no naturesis resulting from 2 cc. of mercuhydrin given intramuscularly alone.

These data may be interpreted as additional evidence that altered aldosterone metabolism plays a part in the salt and water retention in cirrhosis with ascites.

### The Urinary Excretion of 5-Hydroxyindoleacetic Acid After the Administration of Serotonin Precursor in Patients with Hepatic Cirrhosis

By *Robert Donaldson and Seymour J. Gray.* Department of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School.

Preliminary reports from other laboratories have suggested that the metabolism of 5-hydroxytryptamine (5HTA) might be altered in liver disease.

In this study the daily urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) was measured according to the method of Udenfriend et al. in 27 patients with decompensated hepatic cirrhosis and in 40 control subjects without liver or renal disease. The precursor of serotonin, 5-hydroxytryptophan (5-HTP), was administered intravenously in doses of 0.66 mg./Kg. to 11 patients with decompensated cirrhosis of the liver and to 14 control subjects, and the increase in the urinary excretion of 5-HIAA was determined. In each instance the percentage of the administered 5-HTP which was excreted in the urine as 5-HIAA was then calculated. The excretion of 5-hydroxyindoleacetic acid was studied in normal and cirrhotic subjects after the intravenous administration of 0.2 mg./Kg. of 5-HIAA and 5HTA. The urinary excretion of creatinine was determined as an indication of completeness of urine collections.

Although the daily excretion of endogenous 5-HIAA did not appear to be different in control and cirrhotic subjects, there appears to exist in the 11 cirrhotic subjects studied an alteration in the metabolism of exogenously administered serotonin precursor. The mean daily excretion of 5-HIAA was 4.8 mg./24 hrs. in the control subjects compared to 5.3 mg./24 hrs. in the patients with cirrhosis. In the 14 control subjects given serotonin precursor 31% (13–39%) of the administered 5-HTP was excreted in the urine as 5-HIAA in 24 hours. In the 11 patients with cirrhosis, however, 70% (48–100%) of the administered 5-HTP was excreted as 5-HIAA in 24 hours. In both groups most of the increased amount of 5-HIAA appeared in the urine within 6 hours. There was no striking difference in the recovery of 5-HIAA after administration of 5-HTA and 5-HIAA in the normal and cirrhotic groups. Paper chromatographic analyses have revealed no qualitative differences in the indoles excreted by normal and cirrhotic subjects.

### Production of Impending Hepatic Coma by Chlorothiazide and Its Prevention by Antibiotics

By *Joseph E. Mackie, James M. Stormont, Robert M. Hollister and Charles S. Davidson.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston.

Impending hepatic coma (confusion, drowsiness, tremor) developed in 5 alcoholics with cirrhosis and fluid accumulation while given chlorothiazide to promote diuresis. It was prevented by concurrent oral administration of broad spectrum nonabsorbable antibiotics (neomycin and paromomycin).

Measurements of water, electrolyte balance and arterial ammonia concentration were made on 3 of these patients who had shown previous episodic stupor. All were on constant 80 to 100 Gm./day protein diets, and in 2 with marked ascites sodium intake was 200 mg. daily, during 4 to 7-day periods of (1) control; (2) chlorothiazide, 0.5 to 1.0 Gm./day; and (3) chlorothiazide, 1.0 Gm./day plus antibiotic 2.4 to 2.8 Gm./day.

Impending hepatic coma developed in all 3 patients during 8 periods, 24 to 120 hours after beginning chlorothiazide administration. It did not develop during 4 periods when antibiotic was also given. Average arterial ammonia concentrations rose above control values (49 to 101 $\mu$g. %) in all but 1 period with chlorothiazide alone, reaching 245 $\mu$g. % in 1 instance. However, when antibiotic was also given, average arterial ammonia rose slightly and transiently in only 1 period.

In the 3 patients sodium and potassium excretion increased during chlorothiazide administration. The maximum increases in sodium were 37, 107 and 122 and in potassium 72, 63 and 66 mEq./day, respectively. Urine volume increased slightly. In only one patient did serum sodium concentration fall (138 to 130 mEq./L.). Serum potassium concentrations decreased in all instances (below 3.2 mEq./L. in only one patient). In this subject potassium chloride admin-

istration, 6 to 9 Gm./day, rather than antibiotic during 1 period did not prevent tremor; but depression of consciousness was less.

Possible factors responsible for this syndrome under these conditions include hypokalemia, potassium depletion, hyperammonemia, alkalosis, and direct hepatic toxicity. The results indicate that hypokalemia is not the only factor.

### Clinical Studies with Penicillamine in Hepatolenticular Degeneration

By *Marvin J. Seven, Bernard Kliman and Ralph E. Peterson.* National Institute of Arthritis and Metabolic Diseases.

Penicillamine is a breakdown product of penicillin which shows strong copper-binding properties. Preliminary studies in our laboratory and by Walshe have shown that oral administration to patients with hepatolenticular degeneration (HLD) produces a marked enhancement of urinary copper excretion. Further studies have been carried out in 2 brothers with this disease and in one man with hemochromatosis.

Intramuscular administration of short-and long-acting penicillin preparations in large doses in both patients with HLD produced little or no increase in urinary copper output. Oral penicillin preparations also had little effect. Oral penicillamine produced a marked increase in urinary copper, to as much as 6 mg./24 hrs. following a 3 Gm. dose in one patient, and 12 mg./24 hrs. following a 4 Gm. dose in the other. There was no increase in urinary iron excretion. Oral penicillamine in the patient with hemochromatosis caused a slight increase in urinary iron output, as much as following intramuscular penicillin.

Both patients with HLD were treated with oral penicillamine for periods of 7 months. The optimal dosage schedule appeared to be 2 Gm. in 4 divided doses every other day; i.e., 0.5 Gm. QID every other day. Larger doses produced symptoms of anorexia, nausea, gaseous eructations, and drowsiness. No major signs of toxicity were noted with doses up to 5 Gm. daily. One patient showed continued progression of his disease during therapy. The other showed a dramatic clinical improvement, progressing from a bedridden state to complete ability to care for himself and carry on gainful employment as a grocery clerk.

## NEOPLASTIC DISEASE

### Study of the Relationship of Malignant Disease to Dermatomyositis

By *James T. Grace, Jr. and Thomas L. Dao.* Roswell Park Memorial Institute, Buffalo, New York.

Malignant disease frequently accompanies dermatomyositis. The cause of this association is obscure.

This study concerns a young pregnant woman with an acute breast carcinoma who developed dermatomyositis while under observation. Studies prior to the onset of dermatomyositis revealed strong skin sensitivity to an aqueous extract of her own tumor. The positive skin reactions were of the immediate type. Control skin tests with comparable extracts of her normal tissue were negative. The specificity of the reactions and the presence of circulating antibodies were established by passive transfer studies with her serum.

Interruption of pregnancy and ovariectomy produced temporary tumor regression and improvement of the dermatomyositis. Subsequent tumor progression was accompanied by exacerbation of the dermatomyositis.

There is suggestive evidence that dermatomyositis may be related to hypersensitivity. The frequent occurrence of erythema multiforme, erythema nodosum, urticaria and eosinophilia in early stages of the disease may be so interpreted. Curtis et al. suggested that tumor catabolic products may act as an allergen initiating the disease. The findings in this case support this hypothesis. Skin sensitivity to the tumor extract showed that some component of the tumor was allergenic. Thus, if dermatomyositis does have an allergic basis one may logically implicate sensitivity to a product of the neoplasm in this case. Improvement with tumor remission and exacerbation with tumor progression supports this view.

In subsequent studies we found that patients exhibiting unusual local reactions (inflammation, edema, pronounced tenderness, etc.) about their tumors not infrequently showed skin sensitivity to extracts of these tumors. Derma-

tomyositis may thus represent a relatively rare and generalized manifestation of a more common sensitivity reaction in malignant disease.

### Studies on Estradiol-Sensitive Isocitric Dehydrogenase in Human Breast Cancer

By *Vincent P. Hollander and Thelma E. Adamson.* Departments of Internal Medicine and Biochemistry, University of Virginia School of Medicine, Charlottesville. (Aided by a grant from the United States Public Health Service.)

Previous studies from this laboratory have demonstrated the in vitro estradiol-17 B stimulation of isocitric dehydrogenase in human breast and breast cancer. These studies were done by the spectrophotometric method of Villee which measures DPNH formation. The present studies demonstrate that the estradiol sensitive enzyme system can also be demonstrated by α-ketoglutarate formation.

Optimum conditions for a simple assay procedure for estradiol sensitive isocitric dehydrogenase have been developed which measures ketoglutarate production. The supernatants obtained from centrifuging homogenates at 50,000 x g for 45 minutes were incubated at a final concentration of phosphate buffer pH 6.8 0.008 M, $MgCl_2$ 0.008 M, Versene 0.001 M, dl-$Na_3$ isocitrate 0.002 M, and DPN 0.0003 M, for 3 hours at 30 C. Ketoglutarate is estimated spectrophotometrically on the deproteinized reaction mixture. Three out of 8 mammary tissues from radical mastectomy specimens showed significant estradiol-17 β enhancement of the DPN isocitric dehydrogenase. Five out of 17 mammary carcinomas showed significant in vitro sensitivity.

We have not found in vitro sensitivity in a carcinoma associated with an estradiol insensitive breast. The possibility that enzymatic activity in tumor may be due to admixture with breast tissue has been eliminated by finding estradiol sensitivity in metastatic tumor from 3 patients.

A correlation between clinical response to hormonal therapy and in vitro sensitivity would be of obvious clinical utility. Two premenopausal patients with advanced metastatic disease had tumors which failed to show in vitro sensitivity to estradiol-17 β. Oöphorectomy failed to benefit these patients, suggesting that the tumor tissue was not estrogen sensitive in vivo. One premenopausal patient who showed a dramatic remission following oöphorectomy had a metastatic node which demonstrated in vitro sensitivity to estradiol. Further correlative studies of this type are in progress.

### Studies with Amino Acid Analogs in Transplanted Mouse Tumors

By *James F. Holland and Bradley F. Bryant.* Roswell Park Memorial Institute, Buffalo. (Aided by a grant from the National Cancer Institute, National Institutes of Health.)

The importance of aspartic acid and glutamine in pyrimidine and purine biosynthesis, and of glutamic acid in glutamine biosynthesis is recognized. The participation of these amino acids in transamination reactions is established. Since these reactions are prominent in tumor growth, analogs of these compounds are of interest as potential cancer chemotherapeutic agents.

Alpha-methyl aspartic acid, α-methyl glutamic acid and α-methyl glutamine were obtained. These analogs were compared with the parent amino acids in experiments on Krebs 2 carcinoma, Ehrlich's tumor clone 2 and Leukemia 1210, all growing as subcutaneous transplants from ascites tumors in appropriate mice on stock rations. Treatments started 1 day after transplant and continued for 7 days. Tumor weight and carcass weight were determined after sacrifice on the next day. All compounds were given once daily intraperitoneally in 0.5 ml. saline at a dose level of 3 mM/Kg. There was no apparent host toxicity. Some toxic deaths occurred at 4 mM/Kg. of α-methyl aspartic acid.

No substantial difference in tumor weights was observed after treatments with any of the analogs or their parent amino acids. Appreciable variability among experiments was noted. In the several observations on any one compound, however, none produced changes in tumor weight remarkably different from the saline-treated control animals. No enhancement of effect of threshold doses of diazaoxonorleucine was accomplished with concurrent administration of α-methyl glutamine.

### Variations in Modes of Administration of E-39

By *Robert H. Yonemoto, Ralph L. Byron, Jr. and Edward Shanbrom.* Duarte, California.

One of the newer cytostatic agents, ethyleneimine quinone (E-39) was studied in cases of advanced carcinomas and sarcomas which were not amenable to the standard forms of therapy.

Six patients received intra-arterial infusions,

4 patients received intracavitary instillations, and three patients received direct intratumor injection. The intra-arterial instillation was carried out by passing an arterial catheter through the brachial artery into the abdominal aorta, into the celiac axis or hepatic artery, as indicated. The dosage varied from 20 to 100 mg. daily during the initial phase and smaller amounts were administered weekly for maintenance therapy. The total dosage ranged from 7.7 mg./Kg. to 11.8 mg./Kg., given over a 7–13 day period. Severe leukopenia occurred in 4 cases, moderate anemia developed in 6 cases. Thrombocytopenia was seen in 3 cases, but clinical bleeding tendency was observed in only one patient. The bone marrow depression continued for 2 to 3 weeks following cessation of therapy, but was not accompanied by evidence of infection. Severe liver necrosis and death occurred in one patient who received a total of 800 mg. over a period of 7 days, injected directly into the hepatic artery via a catheter. A case of metastatic breast carcinoma with a previous bilateral adrenalectomy went into nonfatal shock after 50 mg. of E-39 was instilled intrapleurally.

One patient demonstrated objective improvement when E-39 was injected into the tumor directly. Three patients showed a lessened tendency to form intracavitary fluid following local instillation of E-39. Objective evidence of tumor regression was seen in 2 cases treated intra-arterially: a case of a Wilm's tumor with massive hepatic metastases and an inoperable carcinoma of the pancreas with liver metastases.

## NERVOUS SYSTEM AND MUSCLE

### The Metabolism of Nerve Regeneration

By *Sven G. Eliasson and Marvin D. Siperstein.*
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The metabolic processes which accompany nerve regeneration have been the subject of only a limited number of investigations. A study of the biochemical events in the cut peripheral nerve was therefore undertaken, using $C^{14}$-labeled intermediates.

The in vitro breakdown of acetate to $CO_2$ and the synthesis of cholesterol and fatty acids by sciatic nerve were followed for periods of from 2 to 32 days following unilateral transection. The uncut contralateral nerve served as a normal control. Normal nerve oxidized acetate to $CO_2$ at relatively rapid rates (6–110 millimicromoles) and synthesized fatty acid in smaller but significant amounts (1–20 millimicromoles). In contrast, however, cholesterol was synthesized in nerves from kittens, but not in adult cat nerves.

The most significant finding from these studies was that within 2 days after injury adult peripheral nerve not only increases its oxidation of acetate, but also acquires the ability to synthetize cholesterol. By the 8th day after injury, the rate of cholesterol synthesis in regenerating nerve approaches that of liver, the most active cholesterolgenic tissue in the body. Furthermore, cholesterolgenesis is most rapid at the presumed site of nerve regeneration, the area adjacent to the proximal side of the injury. Fatty acid synthesis, in contrast to cholesterol, was relatively less influenced by nerve injury.

It seems, therefore, that the appearance of the ability to synthesize cholesterol is to some extent a specific accompaniment of nerve regeneration, and so, may represent a fundamental aspect of the regenerative process.

### An Evaluation of a Medical Geriatric Admission Ward in a General Psychiatric Hospital

By *Fred T. Darvill, Jr. and Charles H. Jones.*
Northern State Hospital, Sedro Woolley, Washington.

To ascertain if elderly committed mental patients could be best managed by medical rather than by psychiatric supervision, a geriatric admission ward was established in July 1956 at Northern State Hospital, a state-supported mental hospital.

All patients 60 years of age and older were admitted to this ward and subjected to a standard admission routine (complete history and physical examination, WBC, hematocrit, BUN, EKG, chest x-ray, serology and urinalysis). Other tests were done if indicated. If any doubt existed in the internist's mind that the mental diagnosis was chronic brain syndrome due to cerebral arteriosclerosis, or to senility, psychiatric consultation was obtained. Patients discovered to have primarily treatable (i.e. nonsenile) psychia-

tric illnesses were transferred to a psychiatric floor for therapy.

The first 100 male patients admitted ranged in age from 60 to 91 (average 74). Eighty of these patients were primarily medical patients (i.e. chronic brain syndromes) and 20 patients were primarily psychiatric patients (mainly involutional psychotic reactions and manic depressive reactions). Ninety-nine different physical diseases were diagnosed in this group of patients; the 5 most common diagnoses were general arteriosclerosis (38), arteriosclerotic heart disease (33), benign prostatic hypertrophy (15), inguinal hernia (13) and malnutrition (13). By December 1957, 30 patients had died, 11 had been sent home, 11 had been discharged to a nursing home and 48 had been transferred to other wards in the hospital. Psychiatric consultation was obtained on 32 patients.

In summary, the above method of handling psychotic geriatric patients allowed prompt treatment of existing medical conditions, adequate screening of elderly patients with treatable psychiatric disorders, improved disposition, and, most important, allowed the psychiatric staff to utilize their time treating the acute mental illnesses of younger patients.

### Structure-Activity Relationships of a New Group of Hallucinogens

By *Adrian M. Ostfeld, Leo G. Abood, John Biel, Ben. Z. Lebovits and Harold Visotsky.* Departments of Preventive Medicine and Psychiatry, University of Illinois College of Medicine, Chicago.

Eleven congeners of the newly-described hallucinogen N-ethyl-3 piperidyl benzylate were synthesized and their psychotomimetic properties and structure-activity relationships examined in rats and in single and double blind studies of 107 humans. When orally administered to humans, .07 to .15 mg./Kg. of 6 congeners induced visual and auditory hallucinations, feelings of unreality, fearfulness, a significant increase in serum ceruloplasmin (p-phenylenediamine method) and changes in the Rorschach, Bender Gestalt, and MMPI psychological tests compatible with a schizophrenic-like state. Behavior changes were assessed by two independent observers, tape-recorded, and photographed.

Compounds which evoked hallucinations in humans induced motor hyperactivity and squealing in rats. All congeners exhibited atropine-like properties in men and rats and on the isolated guinea pig ileum.

The OH group in the benzilic acid moiety and a tertiary rather than quaternary nitrogen linkage were essential to the psychotomimetic effect. Substituting either a cyclopentyl or a cyclohexyl group for one of the phenyl groups of the benzilic acid moiety considerably enhanced hallucinogenic potency, whereas replacement of the phenyl by alkyl groups reduced hallucinogenic effect.

When added to rat brain homogenates, these compounds did not alter the following enzyme activities: cytochrome oxidase, adenosine triphosphatase, choline esterase, and anaerobic glycolysis. Oxidative phosphorylation in rat brain mitochondria was likewise unaffected. Pretreatment of rats with physostigmine abolished peripheral autonomic effects of the compounds without affecting the motor hyperactivity and squealing.

In summary, a new group of synthetic compounds induced a transient psychosis which closely resembled acute schizophrenic reactions in some important psychological and biochemical respects. Synthesis and study of 11 modifications of the basic compound have established the more active hallucinogens and determined the portions of the molecule necessary to such activity. Mechanism of action of these agents seems not to depend on blockade of certain brain enzyme systems or of acetylcholine.

### Changes in Resting Membrane Potential of Skeletal Muscle in the Intact Rat Produced by Closed Arterial Potassium Infusion

By *John C. Harvey and Kenneth L. Zierler.* Department of Medicine, Johns Hopkins University and Hospital, Baltimore. (Aided by contract from Office of Naval Research.)

Hodgkin and Huxley described resting membrane potential of excitable tissues in terms of the Nernst equation, relating ratios of activities of extracellular and intracellular ions, particularly $K^+$ and $Cl^-$. It was our purpose to determine in intact muscle in the living rat how nearly changes in resting membrane potential are determined by changes in extracellular $[K^+]$. To obtain large changes in extracellular $[K^+]$ without killing the rat through cardiac effects, an extracorporeal vena cava was devised, permitting optional diversion of all venous effluent from the leg under study to an external collecter or permitting return to a jugular vein. Blood volume

was maintained by transfusion of normal rat blood into the other jugular vein. Membrane potentials were measured in fibers of the exposed gastrocnemius muscle of anesthetized, respired rats. Local extracellular [K+] was raised by constant infusion of KCl into a cannula placed in the contralateral femoral artery and advanced to the aortic bifurcation. High [K+], thus obtained, was reduced by partial exchange transfusion. Membrane potentials varied with changes in extracellular [K+] in the direction predicted by the Nernst equation, but the changes were quantitatively less than predicted from observed serum [K+]. In part, the departure from theory may have been owing to some increase in intracellular [K+], but in the main it was predictably owing to increased extracellular [Cl−] produced by the infusion.

### Potassium Movement and Muscle Function in Normal Subjects, and in Those with Myasthenia Gravis and Periodic Paralysis

By *David Grob and Richard J. Johns,* Baltimore.

The effect of orally and intra-arterially administered glucose and potassium on movement of glucose, potassium and phosphorus and on muscle responsiveness to nerve stimulation was studied in 12 normal subjects, 8 patients with myasthenia gravis and 2 with periodic paralysis. Glucose administered orally (125 Gm.) or into the brachial artery (Gm.) resulted in approximately the same uptake of glucose by the forearm in each group, but much greater and more prolonged uptake of potassium and slightly greater uptake of phosphorus in patients with periodic paralysis. Glucose had no effect on muscle responsiveness in normal subjects and myasthenic patients, and produced impairment in patients with periodic paralysis, locally after intra-arterial administration and systemically after ingestion. Potassium chloride administration resulted in less uptake of potassium by the forearm in patients with periodic paralysis than in normal or myasthenic subjects, since initial uptake of the ion was followed by loss. It produced a decrease in muscle responsiveness in normal subjects and an increase in patients with myasthenia gravis and periodic paralysis, locally after intra-arterial administration (15 mg.) and systemically after ingestion (11 Gm.). The effect in normal and myasthenic subjects was attributable to enhancement of the depolarizing action of acetylcholine, and in periodic paralysis to this action and to increased contractility, which resulted in extrusion of the ion from muscle, followed by further improvement.

Potassium movement is similar in normal and myasthenic subjects, but administration of the ion improves muscle responsiveness in the latter by antagonizing the acetylcholine-inhibitory block. In periodic paralysis weakness occurs as a result of abnormal uptake of potassium by muscle initiated by the local action of glucose, and recovery occurs when the ion is extruded.

### Electrolyte and Water Content of Skeletal Muscle in Hyponatremia

By *James W. Agna, Lionel R. King and Harvey C. Knowles, Jr.* Metabolism Laboratory, University of Cincinnati College of Medicine, and Cincinnati V. A. Hospital.

There is evidence that cellular composition is altered in hyponatremic states. Accordingly, a study has been conducted of muscle obtained by biopsy from patients with hyponatremia.

Skeletal muscle from 30 subjects was analyzed for water, fat, sodium, potassium, chloride and nitrogen. There were 3 groups: group I, 10 normal subjects; group II, 10 patients with normonatremia and chronic disease; group III, 10 patients with hyponatremia and chronic disease. Plasma electrolyte, water, and specific gravity determinations were performed on specimens obtained at the time of biopsy. The mean plasma concentrations of sodium and potassium expressed in mEq./L. were, respectively, 140 and 4.2 in group I, 139 and 4.3 in group II, and 117 and 4.6 in group III.

The mean potassium concentration of the hyponatremic group (group III), expressed per fat-free solids, was significantly lower than the mean potassium concentrations of group I or group II. The total water contents of all muscle specimens were elevated in the hyponatremic group (group III). According to derived data, the increase in water was related to extracellular expansion. The mean intracellular water contents of all 3 groups were not significantly different, but there was a significant decrease in the mean concentration of potassium expressed per liter of cellular water in group III (120 mEq./L.) as compared to group I (140 mEq./L.) and group II (135 mEq./L.). The cellular sodium content of group III did not differ from group I or group II.

These findings indicate that hyponatremia may be associated with a decreased cellular po-

tassium content. This potassium-deficient state appears to differ from the potassium-deficient states occurring in conditions such as gastroenteric disorders and diabetic acidosis in that hypokalemia and the characteristic clinical findings attributed to potassium deficiency are absent.

## PHARMACOLOGY

### Is Lithium Intoxication Mediated by Potassium?

By *R. Tarail and T. E. Bennett.* Metabolic Section, Roswell Park Memorial Institute, Buffalo.

It is currently held that lithium intoxication causes elevation of serum potassium and cardiac death mediated by potassium. To separate effects of lithium and potassium a blood dialyzer (MacNeill) was used in 17 pentobarbitalized dogs, to compare effects of lithium chloride when elevation of serum potassium is ameliorated with its effects when elevation is encouraged.

Bath fluid LiCl and KCl varied from 15 to 20 mM/Kg. body weight/L. of bath and 0 to 15 mM/L. of bath respectively. Simultaneous electrocardiographic (Lead II), blood pressure, and respiratory tracings were recorded. Serum potassium was measured after equilibration as $K^{42}$; serum lithium was not measured.

Major electrocardiographic changes consistent with potassium effect (prolonged PR, absent P, or wide QRS) ensued in 7 of 9 dogs dialyzed against baths containing lithium with significant loads of potassium. Mean serum potassiums were 3.9 mEq./L. initially and at 1/3, 2/3, and 3/3 of the interval before respiratory arrest 5.7 (1/3), 7.3 (2/3) and 10.3 (3/3). Only minor electrocardiographic changes (P decrease or large negative T) occurred in the two other dogs despite indistinguishable serum potassium elevation. Similar minor electrocardiographic changes (6 dogs) accompanied dialysis against baths containing lithium without potassium; mean serum potassiums were 2.5 mEq./L. initially, 3.2 (1/3), 4.4 (2/3), and 7.4 (3/3). These minor electrocardiographic changes developed also in 2 dogs dialyzed against baths with lithium and 4.2 mM KCl/L. of bath fluid; average serum K was 4.0 mEq./L. initially, 4.0 (1/3), 4.4 (2/3), and 7.1 (3/3).

Thus in 10 of 17 dogs lithium intoxication developed despite minor electrocardiographic changes. Blood pressure was usually maintained before respiratory arrest whether or not major electrocardiographic changes occurred.

These findings suggest a direct effect of lithium on respiration independent of secondary cardiac toxicity of potassium.

## RADIATION

### Reduction of Radiation Hazards in Roentgenography by a New Screen-Film Combination

By *Goffredo G. Gensini.* Department of Research and Laboratories, National Jewish Hospital, Denver.

Recently much emphasis upon the hazards of radiation from diagnostic roentgenography has appeared. The present investigation deals with a new technic which reduces by 15- to 50-fold the hazards of such procedures. Preliminary studies have indicated that the combination of the fastest contemporary photographic emulsion with zinc cadmium sulfide intensifying screens resulted in a very substantial decrease in both the time of exposure and the amount of radiation required. These studies demonstrated, however, that the resolving power of the screens available at the time (CB2 screens for fluoroscopy) was not adequate to reveal the fine details required in many roentgenographic applications. Several experimental screens have been produced, designed to maintain the high efficiency and broad band of spectral emission of the zinc cadmium sulfide screens (CB2) while improving their resolving power. Comparative studies have been performed and particular emphasis has been placed on the speed and resolving power of both the experimental and commercial combinations. Radiation intensity, light emission, optical density and lines of definition per millimeter were measured by physical methods demonstrating the

desirable characteristics of these new screens and films as compared with commercially available screens and films.

### The Postirradiation Syndrome in Humans: Hemopoietic and Lipoprotein Alterations

By *Arvin S. Glicksman, Harry N. Bane, Marion Barclay, Mary L. Petermann and J. J. Nickson.* Departments of Radiation Therapy and Medicine, Memorial Center, and Divisions of Experimental Pathology and Protein Chemistry, Sloan–Kettering Institute, New York. (Aided by a grant from the Research and Development Division, Office of the Surgeon General, Department of the Army and the Kress Foundation.)

Twenty patients, all with cancer, have been studied following treatment with total body irradiation with single doses ranging from 20 to 150 r in air at the midline of the body. This report will deal with changes in formed elements of the blood, lipoprotein alterations, and their relation to the bleeding diathesis seen in the postirradiation syndrome.

There was a decrease in the formed elements of the blood with a sequential fall of lymphocytes, platelets and granulocytes. Maximum depression occurred at about the 4th week after irradiation, in contrast to the reported animal data which show maximal depression at 2 weeks. There have been no consistent alterations in the erythroid or myeloid series of the bone marrow after this dose of total body irradiation.

The changes in serum lipoproteins which appear after therapeutic doses of irradiation are similar in character, although not quantitatively, to the changes seen in rabbits and dogs following lethal radiation. In most patients there was an apparent increase in "clearing activity" during the first week postirradiation. In 6 of the 7 patients who developed evidence of bleeding, there was significantly increased "clearing activity" at the height of the bleeding diathesis.

In 2 patients the problem of bleeding became sufficiently severe to warrant attempts at correction. Because the lipoprotein studies reported here could be indicative of a "circulating heparinoid" substance at the time of the bleeding, and because of animal studies reported by Jacobson and Allan in 1949, intravenous protamine sulfate was administered on 3 occasions to these 2 patients. This resulted in prompt cessation of bleeding as well as correction of the prolonged bleeding and clotting times which were observed.

Therefore, it is felt that evidence exists to suggest that at least part of the bleeding diathesis associated with the postirradiation syndrome may be mediated through a "circulating heparinoid" substance in human beings as well as in experimental animals.

### Effect of Lethal X-irradiation on Tissue Desoxyribonuclease

By *N. B. Kurnick, Barbara W. Massey and Georgianna Sandeen.* V. A. Hospital, Long Beach, California, and Department of Medicine, University of California, Los Angeles. (Aided by a grant from the American Cancer Society.)

The experiments were designed to test our hypothesis that x-irradiation lethality results from inactivation of an inhibitor of acid desoxyribonuclease (DNase), permitting autolysis of chromosomal DNA. Organ weight, DNA content, and DNase activity were measured at intervals after LD100 total body irradiation. Concomitant with massive loss in cell number, spleen, bone marrow, and thymus acid, DNase increased 700 to 2,300% per average cell and to less extent in total organ activity. Liver, which shows much less marked cell loss (about 30%), increases 200% in acid DNase activity per average cell. In animals which received intravenous homologous bone marrow 24 hours postirradiation, bone marrow and spleen recovered by the 7th day, thymus in 30–60 days, while liver did not return to normal. Unprotected animals continued to show elevated DNase activities to death. The data and roughly reciprocal results on inhibitor of alkaline DNase are consistent with the proposed hypothesis of irradiation mechanism, and exclude some alternative hypotheses.

# RESEARCH METHODS

## "Normal Volunteer Subjects": Their Personality Profiles

By *Morton D. Bogdonoff, Joseph J. Combs, Jr., Gerald N. Bryant and James V. Warren.* Department of Medicine, Duke University Medical Center, Durham, North Carolina.

Most of the physiologic data relating to the cardiovascular system in humans have been obtained from observations made of "normal volunteer subjects." Recently, while studying the effect of induced emotional states on cardiovascular function utilizing venous catheterization, we have conducted a series of comprehensive personality interviews of the "volunteers." These interviews have consisted of a biographical account of family and home development, an inventory of feelings and attitudes, and reasons for volunteering.

Twenty "volunteer" male subjects were studied. All were university students who were informed of the experiments either by word-of-mouth communication in the dormitories, or by a bulletin board announcement. A fee was offered. The stated reasons for participating in the study were as follows: in 15 subjects, "need for money"; in four, "interest in science"; in one, "check on my health."

Four subjects presented history patterns indicative of serious personality disorders, two with marked psychoneurotic patterns, and two with depressive illness. In 13 subjects, there was a history of one parent's being an extremely overbearing, aggressive individual, and these subjects universally reported the inability adequately to express their own feelings of hostility and aggression. Two of these individuals indicated a "need to experience pain and toughen up." Two subjects reported volunteering for these experiments shortly after undergoing intensely stressful difficulties in life adjustment. Six reported home backgrounds characterized by elements of intense discord. Life histories of 5 of the subjects appeared unremarkable.

The incidence of personality adjustment problems in "normal volunteer subjects" indicates that in the study of relationships between affect state and physiologic changes, the nature of the subject group must be taken into consideration.

## Is Lymph Measured as a Part of Plasma Volume?

By *Robert L. Funkhouser and Walter H. Pritchard.* Department of Medicine, University Hospitals, and Western Reserve University School of Medicine, Cleveland. (Aided by a grant from the Cleveland Area Heart Society.)

According to one current hypothesis, the greater size of the blood volume estimated with labeled serum protein over that estimated with labeled red cells is to be explained by loss of labeled albumin into the extravascular lymphatic spaces. If this is the explanation, the loss must be soon after injection, for after the "'mixing curve" is complete, losses are accounted for by the usual extrapolation.

In an attempt to measure experimentally rapid lymphatic protein losses known to occur in the liver, 7 dogs with chronic constriction of the inferior vena cava and ascites were subjected to cannulation of the liver lymphatics after a series of blood volume determinations with $Cr^{51}$-labeled red cells and $I^{131}$-labeled canine plasma protein. The appearance of labeled protein in the lymph was followed, comparison being made with 5 dogs who had no venous constriction, but who had been similarly treated in other respects.

The rate of appearance of labeled protein in liver lymph in the dogs with hepatic venous congestion varied from values similar to those found in control animals to enormously increased values in 2 dogs whose lymph radioactivity was at equilibrium with plasma radioactivity 25 to 30 minutes after injection. In these dogs, then, an estimate of plasma volume based on extrapolation of plasma radioactivity after 30 minutes from the time of injection would have included the majority of the liver lymph, but in a 10–30 minute period of extrapolation, the slope of decline would have corrected for this demonstrated loss of tagged albumin.

From these studies it is suggested that only in exceptional circumstances is liver lymph included in estimates of plasma volume with labeled protein, although the liver is one of the sites of the most rapid extravascular protein turnover known.

# RESPIRATORY SYSTEM

## Studies of Free Collapse in the Intact Human Lung

By *John A. Pierce.* Department of Medicine, University of Arkansas Medical Center, Little Rock.

The purpose of this study was to determine the pattern of expiratory air flow during free collapse of the lungs in normal humans. It has been assumed that inertia is negligible following the peak volume flow rate. Hence the rate of lung collapse will be a function of the elastic properties of the lung and the resistance to movement of the lung.

An intraesophageal balloon was utilized as an index to intrathoracic pressure. Following maximal inspiration, the subjects expired into a special spirometer which has virtually no resistance. A little practice with the balloon in place permitted the subjects rapidly to approximate atmospheric pressure within the chest. The resulting curves were recorded photographically. They included the intraesophageal pressure, the volume of flow and the volume flow rate. The relationship between volume and time was exponential above the level of pulmonary mid-capacity. The mean half time was 0.46 ($\pm$0.13) seconds. A knowledge of the statically measured pressure-volume relationships over the entire range of lung inflation permitted calculation of the mean resistance to air flow. In 42 such curves from 7 healthy subjects it was 2.49 ($\pm$0.37) cm. $H_2O$/L./sec.

The generally accepted equation for airway resistance is: $P = k_1\dot{v} + k_2\dot{v}^2$, where P is pressure, $\dot{v}$ is rate of air flow and $k_1$ and $k_2$ are constants. The initial term on the right reflects laminar flow while the final term denotes turbulent flow. Expiratory air flow patterns have been predicted from this equation using the measured values for $k_1$ and $k_2$ in these subjects.

The results indicate that the curve of expiratory air flow during free collapse of the lungs closely approximates the predicted curve based on the assumption that flow is completely laminar. It appears that whatever resistance is contributed by turbulent air flow is offset by an increase in the size of the bronchi at high levels of lung inflation.

## Effects of Bilateral Cerebral Infarction on Respiratory Center Sensitivity and Arterial Blood Gases

By *H. O. Sieker, R. I. Birchfield and A. Heyman.* Department of Medicine, Duke University School of Medicine, and V. A. Hospital, Durham, North Carolina.

The study was undertaken to determine the influence of the cerebral cortex on the respiratory center and the regulation of pulmonary ventilation.

Alterations in pulmonary ventilation, arterial pH, oxygen saturation and carbon dioxide tension was studied in 9 patients with bilateral cerebral infarction. Seven of these patients showed various types of periodic breathing. The sensitivity of the respiratory center was determined by the ventilatory response to inhalation of carbon dioxide. The changes in respiratory function in these patients were compared with those in 9 control subjects of the same age and 7 patients with a single or unilateral cerebral infarction.

The mean arterial carbon dioxide tension in the patients with bilateral infarction was 41 mm. Hg, a value significantly less than that of 45 mm. Hg observed in the control subjects ($p < .001$). The oxygen saturation and minute ventilation were comparable in all groups, but the patients with bilateral cerebral damage had a significantly higher arterial pH ($p < .02$). These patients also showed a marked sensitivity to carbon dioxide inhalation with a striking increase in ventilation of 235% as compared with an increase of 141% in the control subjects. The patients with unilateral infarction generally showed changes in respiratory function intermediate between the normal subjects and those with bilateral brain damage.

These studies indicate that bilateral cortical damage alters the chemical regulatory mechanisms of respiration and permits the respiratory center to operate at a lower controlling level of carbon dioxide tension. The respiratory center when released from cortical control develops an exaggerated response to chemical stimuli.

## Effect of Acutely Induced Metabolic Acidosis on the Respiratory Response to $CO_2$ in Normal Man

By *James K. Alexander and Junichi Mise.* Baylor University College of Medicine, Houston.

To investigate the effects of acute changes in blood hydrogen ion concentration upon the ventilatory response to $CO_2$ inhalation in man, observations have been made before and after

induction of metabolic acidosis by infusion of 2% ammonium chloride solution. Simultaneous measurements of ventilation together with arterial blood pH and $pCO_2$ during inhalation of varying $CO_2$ concentrations in the inspired air permitted determination of stimulus–response curves relating change in ventilation to change in blood hydrogen ion concentration or $pCO_2$.

In 9 normal subjects studies were carried out under basal conditions before and after infusion of ammonium chloride solution sufficient to lower arterial blood pH 0.02–0.04 units. Ventilation tended to rise only slightly or not at all after ammonium chloride infusion while breathing room air, but with 3% $CO_2$ and 5% $CO_2$ inhalation ventilatory volume was greater after ammonium chloride administration. Change in alveolar ventilation and total pulmonary ventilation per unit change in arterial blood $pCO_2$ was increased after induction of acidosis. However, no change could be demonstrated in the relationship between ventilatory response and arterial blood hydrogen ion concentration after ammonium chloride. It is tentatively concluded that in normal subjects the sensitivity of the respiratory regulatory mechanism to $CO_2$ is increased with development of acute acidosis.

### Effect of Salicylates upon the Ventilatory Response to Carbon Dioxide in Normal Subjects

By *Philip Samet, Eugene M. Fierer and William H. Bernstein.* Cardio-Pulmonary Laboratory and Department of Medicine, Mount Sinai Hospital, Miami Beach, and Section of Cardiology of the Department of Medicine, University of Miami School of Medicine, Coral Gables, Florida.

Previous studies in this laboratory have demonstrated that salicylate administration does not produce an increased ventilatory response to inhaled carbon dioxide in subjects with obstructive pulmonary emphysema and hypercapnia.

The purpose of this study is to evaluate the effect of salicylates upon the ventilatory response to carbon dioxide in normal subjects. Eleven patients were studied. Minute ventilation, alveolar ventilation, oxygen consumption, respiratory quotient, respiratory frequency, tidal volume, and arterial blood oxygen saturation, carbon dioxide tension and pH were determined during inhalation of compressed air, 3% $CO_2$ in compressed air and 5% $CO_2$ in compressed air before and 1½–3 hours after salicylate ingestion. Blood salicylate levels ranged from 11 to 19 mg.%.

Minute and alveolar ventilation increased 48% and 89% respectively in response to 3% $CO_2$ inhalation. The corresponding figures after salicylate administration were 69% and 97%. Minute and alveolar ventilation increased 178% and 270%, respectively, during 5% $CO_2$ breathing. The corresponding figures after salicylate ingestion were 239% and 276%. These data suggested that most of the increase in minute ventilation was due to increased dead space ventilation with a relatively small increase in alveolar ventilation.

This concept was further supported by the arterial blood carbon dioxide tension data. The latter were 41, 45 and 48 mm. Hg during inhalation of compressed air, 3% $CO_2$ and 5% $CO_2$, respectively. After salicylate administration, the corresponding figures were 41, 44 and 49 mm. Hg. Arterial blood pH during inhalation of the 3 gas mixtures was also unchanged by salicylate administration.

### Maximum Expiratory Flow Rate: A Useful, Easily Performed Respiratory Test

By *Harold W. March and Harold A. Lyons.* Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn.

A 2-year laboratory study of the application of maximal expiratory flow curves in the estimation of ventilatory capacity in restrictive and obstructive disease has been made. Normal curves have been obtained in men and women of 2 age groups, 20–35 years, and 45–66 years. Patients with pulmonary sarcoidosis illustrate the effect of restrictive disease on the forced expiration curve, and a group of emphysema patients demonstrate the changes wrought by airway obstruction.

In general, normal values tend to decrease with age and sex so that the mean flow rate in young men was 477 L./min. and in elderly women, 180 L./min. In restrictive disease the form of the curve is usually well maintained, but there is a reduction in flow commensurate with the degree of restriction as expressed by the vital capacity. In emphysema, marked reductions in flow occur and the form of the curve is profoundly altered. In addition to the effect of reduced vital capacity, many of these patients demonstrate a specific mechanism of airway closure which abruptly checks expiratory flow. In emphysema, a "point of closure" is noted which separates a short period of relatively fast flow from a long segment in which volume and flow are minimal.

The maximal expiratory flow rate is a useful test of ventilatory function. It has the advantage of rapidity and simplicity of performance and also the desirable feature of minimizing the voluntary cooperation of debilitated or poorly motivated patients. The standard values give information similar to the maximum breathing capacity.

### Intrapulmonary Gas Distribution during Hypothermia and upon Rewarming

By *Emil Blair and James Fellows.* U. S. Army Research and Development Unit, Fitzsimons Army Hospital, Denver.

Aside from studies of oxygen consumption and minute ventilation, relatively little investigation on pulmonary function during hypothermia has been reported. The present study is concerned with intrapulmonary gas distribution during varying periods of hypothermia and after rewarming. Mongrel dogs under chloralose anesthesia with unassisted ventilation were used. The intrapulmonary gas distribution and functional residual capacity (FRC) were determined by the helium gas open-circuit method. The study was divided as follows: (a) control normothermia to 6 hours of anesthesia; (b) hypothermia (31 to 26 C.); (c) rewarm; (d) normothermia with controlled low ventilation rates (curarization and respirator).

*Results:* (a) Normothermia; with longer anesthesia periods, uneven mixing and slightly increased FRC developed. There was no consistent change in minute ventilation or in respiratory rate. (b) Hypothermia; there was a pronounced increase in defective distribution with little alteration in FRC and a marked depression in minute volume and in rate. With prolonged hypothermia, gas distribution improved and FRC remained unchanged. Minute ventilation and rate increased but not to normothermic levels. (c) Rewarm; return to control levels except FRC which tended to remain somewhat increased. (d) Normothermia controlled-low minute ventilation and rate. Gas distribution (compared with normothermia a) became more defective, along with no significant alteration in FRC.

The increased degree of defective gas distribution during hypothermia appeared to be due to reduced over-all ventilation rates, the latter likely resulting from central respiratory depression. Reduced over-all ventilation rates during normothermia produced similar changes. As the period of hypothermia progressed (4 hours observation), the over-all ventilation improved and, concomitantly, gas distribution. Hypothermia apparently does not result in any appreciable lung changes to the extent determinable by study of intrapulmonary gas mixing.

### A Rebreathing Method of Measuring Pulmonary Diffusing Capacity for Carbon Monoxide

By *Benjamin M. Lewis, Tai-hon Lin and Frances E. Noe.* Department of Medicine, Wayne State University College of Medicine, and City of Detroit Receiving Hospital.

The single breath technic of measuring pulmonary diffusing capacity for carbon monoxide ($D_{L_{CO}}$) assumes that alveolar CO concentration falls exponentially with time. This assumption is incorrect even in normals (Forster et al. 1954), a fact we have confirmed in 3 instances. To circumvent this difficulty we have adapted a rebreathing method using $C^{14}O$ (Kruhøffer, 1954) to the use of stable CO. The subject expires to residual volume and then rebreathes rapidly from a bag containing a volume of 0.3% CO about equal to his vital capacity. CO concentration is analyzed continuously by cycling a portion of the gas in the bag through an infra-red CO meter and then returning it to the bag. We have invariably obtained an exponential fall of CO concentration with time after the first 10 seconds in subjects with and without pulmonary disease. From this exponential fall we have computed $D_{L_{CO}}$ in 13 normals; our average value was 22.76 ml./mm.Hg/min. In the same group the single breath $D_{L_{CO}}$ using 10-second breath-holding averaged 27.0 ml./mm.Hg/min. Five determinations in 4 patients with pulmonary sarcoidosis gave an average rebreathing $D_{L_{CO}}$ of 14.8 ml./mm.Hg/min.; the average single breath $D_{L_{CO}}$ was 17.6 ml./mm.Hg/min. The correlation between the 2 methods was high ($r = .923$). In 3 patients with severe emphysema the rebreathing $D_{L_{CO}}$ averaged 16.7 ml./mm.Hg/min.; in only one of these could a satisfactory single breath $D_{L_{CO}}$ be measured; it was 10.6 ml./mm.Hg/min. The rebreathing method has the following advantages: CO concentration falls exponentially with time; the method can be used in all patients; it is simple and rapid; it incorporates an internal check on the analysis of CO and, if 10% helium is added to the mixture, an internal check on the volume of the system as well.

### The Effect of Altering Lung Volume on Pulmonary Diffusion of Carbon Monoxide

By *Birger Grape and John M. Tyler.* Lemuel Shattuck Hospital, Harvard Medical School, and Harvard School of Public Health. (Aided by a grant from USPHS.)

Since the "single breath" method for determining the diffusion of carbon monoxide, ($D_{L_{CO}}$), is carried out at maximal inspiration and the "steady state" method at resting mid-position, this disparity in alveolar volume, ($V_A$), might partly explain the higher normal values found by the first method.

The influence of altering $V_A$, produced by changing transpulmonary pressure, on $D_{L_{CO}}$ was studied 10 times in 5 normal subjects and in one patient with restrictive pulmonary disease. A tank respirator was modified to maintain unvarying pressure. "Steady state" determinations of $D_{L_{CO}}$ were carried out at +10 and −10 cm. $H_2O$ intratank pressure. The mean $V_A$ change was .88 L. End-tidal $pCO_2$, obtained with a rapid analyzer, was used to calculate the dead space. In the one subject tested, $Pa_{CO_2} = PA_{CO_2}$ at both levels of $V_A$. (In 18 consecutive subjects at ambient pressure $Pa_{CO_2} - PA_{CO_2} = 1.4$ mm. Hg ± 1.1 (1 SD).) In all paired instances $D_{L_{CO}}$ increased with $V_A$, mean rise 8.0 ± 2.3 cc./min./mm.Hg/L. $V_A$ increase. One subject with an extremely steep rise was excluded. $P_{A_{CO}}$ declined .035 ± .017 mm. Hg. There was no significant change in minute ventilation, dead space, or CO uptake at the two levels of $V_A$.

Parallel dead space would be missed by this technic. If present, it would increase the change with $V_A$. The error of ignoring back pressure would make the true $D_{L_{CO}}$ higher at the larger $V_A$ when this was run following the smaller $V_A$. In two experiments the order was reversed without changing the findings.

In conclusion, it has been shown that $D_{L_{CO}}$ varies with $V_A$. The effect of changing transpulmonary pressure on ventilation-perfusion relationships was not assessed. An increase in $V_A$ probably increases the diffusing area and this might account for the above results and also explain the difference between the "single breath" and the "steady state" method for determining the diffusion of carbon monoxide.

### Analysis of Factors Influencing Alveolo-capillary Oxygen Diffusion in the Lung

By *Peter C. Luchsinger, Kenneth M. Moser and Sol Katz.* Cardio-Pulmonary Function Laboratory, District of Columbia General Hospital, and Department of Medicine, Georgetown University Medical School, Washington, D. C.

Many use the terms "low diffusing capacity" and "diffusion insufficiency" interchangeably, implying that both describe the same physiologic defect.

Studies using combined hemodynamic and pulmonary investigative technics will be presented which demonstrate that: (1) a diffusion insufficiency is characterized by a widened alveoloendcapillary $pO_2$ gradient (reflected in a widened alveolo-arterial oxygen tension gradient) and can exist whether the diffusing capacity is decreased or normal; (2) a low diffusing capacity may be present without diffusion insufficiency; (3) diffusion insufficiency is commonly associated with a fixed and usually elevated pulmonary vascular resistance. This leads to pulmonary hypertension during exercise and, when severe, at rest.

These observations indicate that diffusion insufficiency is related to a decrease in size of the pulmonary capillary bed. Such reduction leads to an increase in pressure at the entrance to the pulmonary capillary bed and results in a reduced contact time between red cell and alveolar oxygen tension.

While alveolo-capillary diffusion defects have previously been felt to reflect chiefly the presence of a thickened "membrane," these results indicate that "contact time" is in many instances of major importance. Furthermore, while there is a gross relationship between a low diffusing capacity and diffusion insufficiency, the abnormalities described by these terms are not identical and each should be reserved for its own spectrum of proper usage.

### Hemodynamic Effects of Pressure Breathing

By *Kaye H. Kilburn, Herbert O. Sieker and Herschel V. Murdaugh, Jr.* Department of Medicine, Duke University Hospital, and V. A. Hospital, Durham, North Carolina.

Knowledge of the effects of alterations in intrathoracic dynamics produced by pressure breathing on renal excretion of water and electrolytes prompted a study of the effects of these alterations on cardiac output, stroke volume and plasma volume. The cardiac output of 18 normal subjects was measured by recording indicator-dilution curves after peripheral or central injection of T-1824 on a cuvette densitometer before and during negative pressure breathing (18–22 mm. Hg). Plasma volumes were measured by a dilution equilibrium method using $I^{131}$-labeled

human albumin in 5 subjects. Urine flows were also measured.

Negative pressure breathing increased the cardiac index from a control level of 2.85 ± 0.18 (S.E.) L./min./sq.m. to 3.98 ± 0.30(S.E.) L./min./sq.ml. and increased the stroke volume from 68.3 ± 4.3(S.E.)ml. to 95.4 ± 9.3(S.E.) ml. There was no significant change in total plasma volume. Urine flow was consistently increased from 1.21 ml./min. (control) to 6.49 ml./min. on negative-pressure breathing.

Conversely, positive-pressure breathing produces a small decrease in cardiac index and stroke volume and in total plasma volume.

The evidence suggests that the increase in urine flow produced by negative pressure breathing is accompanied by an increase in cardiac output, but is not accompanied by an increase in total plasma volume. The evidence implies that the exaggerated cyclic variations in intravascular and intracardiac pressures produced by pressure breathing triggers the afferent limb of a urine volume control system.

### The Cardiopulmonary Response of Anemic Subjects to Heavy Exercise on a Treadmill

By *Brian J. Sproule, Jere Mitchell and Carleton B. Chapman.* Cardiopulmonary Laboratory, Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

The cardiopulmonary response of 10 anemic male subjects (Hb less than 10 Gm.) to heavy exercise was studied by collecting expired air and analyzing blood samples drawn at rest and during exercise from brachial artery, brachial vein, and femoral vein for oxygen content, capacity, $pO_2$ (polarographic), carbon dioxide content, pH and $pCO_2$ (calculated).

Maximal oxygen intake was less than in 30 similarly studied normal subjects although equivalent workloads were achieved. At rest, oxygen transport to body tissues (1029 cc./min.) was normal; cardiac index (4.67 L./min.), central blood volume (1.94 L.), stroke volume (87.8 cc.) and minute ventilation (12.52 L./min.) were increased. At maximal exercise, cardiac index (13.45 L./min.), central blood volume (3.106 L.) and stroke volume (136.7 cc.) were normal although minute ventilation (73.45 L./min.) and tidal volume (1780 cc.) were less than in normal subjects.

The percentage of oxygen utilized was the same as in normal subjects, both at rest (36.29%) and at exercise (75.11%). $AVO_2$ difference increased, as in normal subjects, 2.17 times and $pO_2$ did not change significantly from rest to exercise in either venous or arterial blood although venous oxygen content fell. The data suggest a shift to the right of the $O_2$ dissociation curve in venous and arterial blood at both rest and exercise. The A-a $O_2$ transfer gradient was increased at rest (39.16 mm. Hg) and did not change on exercise.

The change from rest to exercise in both arterial (7.39 to 7.30) and venous (7.37 to 7.19) pH was less in anemic than in normal subjects as was the change of $AVCO_2$ difference (3.98 VPC at rest to 8.30 at exercise).

The study indicates that moderately anemic individuals react to heavy exercise qualitatively and quantitatively as do normals, but compensatory mechanisms are significantly modified by decreased oxygen-carrying capacity.

### The Correlation of Pulmonary Ventilation and Energy Expenditure

By *Amasa B. Ford and Herman K. Hellerstein.* Department of Medicine, University Hospitals of Cleveland, Cleveland.

Pulmonary ventilation is adjusted to the energy expenditure of the body so precisely that one may be predicted from the other. Analysis of 272 observations of working energy expenditure by 52 normal factory and steel mill workers indicates that: $Y = 0.205X - 0.52$, where X = ventilation in liters per minute (STPD) and Y = energy expenditure in calories per minute. The standard error of estimate for a single observation was 0.42 calories per minute.

A hot, humid working environment (heat stress up to 300 on the Belding-Hatch scale) or the presence of currently asymptomatic compensated hypertensive or arteriosclerotic heart disease did not significantly alter the relationship between ventilation and energy expenditure, judging from 42 and 73 observations, respectively. A statistically significant ($p = <.01$) shift of the regression curve to the right was observed in men in the 18–30-year age range and in men whose surface area exceeded 2.05 square meters. This greater "respiratory efficiency" has been observed during treadmill exercise by young men in our laboratory. It is postulated that this difference may be due to changing mechanics during various types of work or to the state of physical conditioning.

### Work of Breathing and Respiratory Control in Obesity

By *J. Howland Auchincloss, Jr., Robert Gilbert and Robert H. Eich.* Department of Medicine, State University of New York Upstate Medical Center at Syracuse, and V. A. Hospital, Syracuse. (Aided by a grant from USPHS.)

Alveolar hypoventilation manifested by $CO_2$ retention, hypoxia, and polycythemia is occasionally found in obese individuals and suggests that obesity affects respiratory control either by increasing the work of breathing or by some other mechanism. Twelve obese subjects were studied. One patient manifested this syndrome with average $P_aco_2$ of 56 mm.Hg.

Using the open circuit method with voluntary hyperventilation, elevated oxygen cost of breathing was found in 4 of 8 subjects; in 3 of these both elastic and non-elastic resistance was measured by the esophageal balloon technic and found to be normal; however, in 3 other patients elastic resistance was normal, but non-elastic resistance was increased. Both oxygen cost of breathing and esophageal balloon studies were normal in the patient with severe hypoventilation.

Total ventilation with respect to body height was elevated in 8 of the 12 patients, but $P_aco_2$ was 45 mm.Hg or more in 3 of these and in 6 of the total 12, and in no case was lower than 39 mm.Hg. Thus elevated resting ventilation, when present, was compensatory to the increased metabolism, and such compensation was not always complete. A defective respiratory response to $CO_2$ was found in 3 of the obese subjects and was also found in one healthy, non-obese, young male with a $P_aco_2$ of 49 mm.Hg on repeated determinations.

The study suggests that the work of breathing need not be elevated for severe hypoventilation to develop in obesity. The obese state produces an increased requirement for gas exchange related to metabolism and probably also to body gas stores. Whether or not the syndrome of hypoventilation, hypoxia, and polycythemia will develop appears to depend upon the relationship between this increased need and the inherent sensitivity of the respiratory center.

### The Relationship of Mood, Dyspnea and Pulmonary Function in Respiratory Cripples

By *F. Gerald Gleeson, Jr., C. J. Martin, Thomas H. Holmes and Allan C. Young.* Firland Sanatorium, and Departments of Psychiatry and Physiology and Biophysics, University of Washington Medical School, Seattle. (Aided by a grant from the National Tuberculosis Association.)

Patients suffering from emphysema complain from time to time of increased dyspnea. Pulmonary function tests (maximal expiratory flow rate, vital capacity, expiratory reserve, alveolar carbon dioxide concentration, carbon dioxide production, nitrogen index, dead space, and volume of alveolar ventilation) were repeated 2 to 3 times a week over a 2-month period on 7 subjects with chronic obstructive emphysema. The findings for each day were related to the difficulty in breathing and the subject's mood.

These data indicate that for a given subject the amount of daily variation in function tests is as large or larger than that expected in normal subjects. Patients with severe functional impairment exhibited as much absolute variation as those with minimal impairment. Using these tests to measure changes in the functional status of such subjects requires that absolute values be considered, since % changes may lead to serious error.

"Mood" and "increased dyspnea" appear to be interchangeable terms in these respiratory cripples. In each patient increased dyspnea was reported on and only on days when mood was described as poor. Pulmonary function tests on the "poor" days when compared with "average" or "good" days did not show significant differences in any of the parameters measured.

As resting functional tests did not distinguish the poor days, the respiratory response to stress was evaluated. In the second part of the experiment function studies were measured before, during and after the introduction of a physical or psychic stress. Mood and level of dyspnea were recorded. One stress situation involved a standard walking exercise and the other a pressure-induced headache.

Comparing the functional data obtained on average days with those obtained on the more dyspneic days indicated no difference in the respiratory response to exercise. The response to the pressure headache on dyspneic or "poor mood" days when compared with average or good days showed a significant decrease in alveolar ventilation and an increase in the alveolar concentration of carbon dioxide. This hypoventilation was accomplished without significant change in the carbon dioxide production. Hypoventilation in these subjects was also noted in extreme emotional situations, anger in one case, and depression in another.

### Blood Ammonia in Pulmonary Emphysema

By *Robert Dutton, Jr., William Nicholas, Curtis J. Fisher and Attilio D. Renzetti, Jr.* Department of Medicine, State University of New York, Upstate Medical Center, and V. A. Hospital, Syracuse.

The occurrence of elevated blood ammonia, confusion and "flapping tremor" in patients with severe pulmonary emphysema has stimulated a study of the incidence and significance of an elevated blood ammonia in pulmonary emphysema without congestive heart failure.

Twenty-four patients with pulmonary emphysema were studied. Of these, 16 had normal liver function as evidenced by normal bromsulfalein clearance and absence of clinical signs of hepatic disease. Venous blood ammonia, as determined by a modification of the Conway method, in these 16 patients averaged 43 gamma % (S.D. $\pm$ 13). Eight had levels above 50 gamma %. The average value in 28 normals was 27 gamma % (S.D. $\pm$ 11). The difference between these groups is statistically significant ($P < .01$). The average blood ammonia in the 8 patients with retention of BSP was 58 gamma %.

Serial blood ammonia determinations were performed on 10 patients. A marked fall was noted in 7 as their clinical status improved.

Pulmonary function studies including arterial blood analysis were performed on 8 patients who regained a stable state after recovery from acute illness. Six had an arterial oxygen saturation of 90% or below. Of these, 5 had a venous blood ammonia in excess of 50 gamma %.

The data suggest that in the absence of hepatic disease or congestive heart failure, pulmonary disease is frequently associated with elevated blood ammonia. Although not conclusive, the data also indicate that greater elevation of ammonia may be found in patients with more severe emphysema.

### Treatment of Pulmonary Emphysema with a Potent Carbonic Anhydrase Inhibitor

By *W. T. Thompson, Jr. and D. W. Richardson.* Medical Service, V. A. Hospital, and Department of Medicine, Medical College of Virginia, Richmond.

Published considerations of the effects of Diamox on alveolar ventilation in respiratory acidosis disagree regarding the degree, duration, and mechanism of the drug's action. Availability of a more potent carbonic anhydrase inhibitor, dichlorphenamide, led to investigation of its effects in 8 patients with emphysema and $CO_2$ retention.

Following achievement of maximum benefit from antibiotics, bronchodilators, and digitalis, dichlorphenamide, 100–200 mg. daily for 10 days, was administered to patients eating a fixed diet (40 mEq.Na, P 85, C 245, F 95). Standard methods were used for all analyses.

Before dichlorphenamide, average arterial values were: $CO_2$ tension ($pCO_2$) 56 mm.Hg; $CO_2$ content 27.8 mM/L.; pH 7.38; and $O_2$ saturation 78.9%. After 36–48 hours of drug administration, average $pCO_2$ decreased 12% ($P < .01$); $CO_2$ content fell 18% ($P < .01$). Mean pH fell .06 units ($P < .01$), and average $O_2$ saturation rose 4% ($P < .05$). On the 10th day, despite further fall in $CO_2$ content, pH remained stable because of continued reduction in $pCO_2$. On the 10th day, average arterial oxygen saturation had risen 10% above its control average. Weight loss, negative water balance and increased excretion of Na, K, $HCO_3$ were noted. Serum sodium was unchanged, potassium decreased slightly and chloride rose significantly. Definite clinical improvement, with disappearance of wheezing and lessened dyspnea and cough, occurred in all patients. One week after the drug was stopped, all laboratory findings returned in the direction of, but did not reach, pre-treatment levels. Clinical improvement continued for several weeks.

Reduction of serum bicarbonate, produced by inhibition of the carbonic-anhydrase-dependent mechanism for renal bicarbonate reabsorption, was accompanied by lowering of arterial $CO_2$ tension. A deepening acidosis was thereby avoided. Concomitant increase in arterial oxygen content suggests that improved alveolar ventilation accounts for the reduced arterial $pCO_2$.

Dichlorphenamide seems a valuable agent in the therapy of respiratory acidosis.

### The Relaxation-Pressure Curve in Restrictive Pulmonary Disease

By *Er Yi Ting and Harold A. Lyons.* Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn.

The relaxation-pressure diagram for normal human subjects has been studied and found to be a good method for obtaining knowledge of the mechanics of respiration. No previous reports of its use in the study of pulmonary disease have been made.

The method of Rahn was employed in the study of these patients with intraesophageal pres-

sure measured by means of an esophageal balloon connected to a water manometer, and air intranasal catheter for the measurement of alveolar pressure. The volume was recorded by a spirometer, and measurements were started at full inspiratory capacity and records made of the intraesophageal pressure, then the intra-alveolar pressure at moments of complete relaxation and decreasing increments of inflation until the expiratory reserve volume was reached. These points of measurement were then diagrammed as the pressure-volume relaxation curve for each subject.

In 12 patients with restrictive pulmonary disease it was found that the pressure-volume diagram was markedly different from that of normal subjects. The curves were flattened and shifted downward and to the right. It can be shown that these findings may be due to one of two factors or a combination of these: (1) alteration of the lung pressure ($P_L$) when the disease is parenchymal, or (2) alteration of the chest wall curve, when the thoracic wall disease is present. The reduction in pulmonary compliance can be assigned to either of these factors from the analysis of the pressure-volume relaxation diagram.

The results show that restrictive pulmonary disease may result from either changes in lung tension, or chest wall pressure change. Both factors may contribute to the abnormality of the respiratory mechanics. This method of study gives greater information than the usual method of measuring pulmonary compliance.

### The Mechanics of Respiration in Normal Pregnant Women

By *Mortimer E. Bader, Richard A. Bader and Michael Truppin.* Mount Sinai Hospital, New York. Aided by a grant from the USPHS.)

Previous investigation of dyspnea occurring so commonly in normal pregnant women has failed to reveal any significant abnormality in conventional pulmonary function tests. Furthermore, there was no correlation of the dyspnea with either breathing reserve or walking dyspnea index.

Study in this laboratory has revealed an increase in the oxygen cost of hyperventilation in normal pregnancy, comparable to that seen in pathologic conditions such as emphysema and mitral stenosis. To determine the reason for the increased energy requirement a study was made of the mechanics of respiration in normal pregnancy.

Pulmonary compliance, pulmonary airway resistance, vital capacity and maximum breathing capacity were measured in 28 normal pregnant women with dyspnea in the 6th to 9th months of gestation, who were free of any cardiac or pulmonary disease, and in 20 normal nonpregnant females. Values for the vital capacity were ± 15% of predicted for both the control and pregnant women. Maximum breathing capacity was ± 17% of predicted for both groups. Mean pulmonary compliance was 0.162 ± 0.063 L./cm. $H_2O$ in the pregnant cases compared with 0.165 ± 0.080 L./cm. $H_2O$ in the control group. Average values for pulmonary airway resistance were 1.51 cm./L./sec. in the pregnant females compared with 1.65 in the controls.

Since the increase in the oxygen cost of breathing cannot be related to significant alteration in the pulmonary compliance, airway resistance, vital capacity or maximum breathing capacity, it would appear that extra-pulmonic factors account for the increase in the oxygen cost as well as the dyspnea of pregnancy. This also lends some support to the view that an increase in the work of breathing may play a role in the dyspnea of pulmonary or cardiac origin.

### Assessment and Comparison of Technics for Obtaining Sputum for Cytologic Examination

By *S. Axelrod, M. Garrett, L. Leilop and H. A. Lyons.* State University of New York.

A survey is being carried out in an attempt to assess the relative merits of symptomatologic, radiologic and cytologic studies in the screening of population groups for lung cancer. There is as yet, no unanimity regarding the relative value of each method applied to an apparently healthy population, as may be found, for example, in industry. A successful and simple technic for obtaining sputum from healthy and ill persons who are otherwise unable to produce a specimen for cytologic examination is an essential prerequisite for the survey, and would also have wider clinical application.

A method has been devised by Bickerman and Sproul which uses a warmed aerosolised solution of hypertonic saline and propylene glycol. It is claimed that the method is both comfortable and innocuous; that a skilled technician is not required; that satisfactory specimens of sputum may be obtained in approximately 88% of well and ill subjects; and that the cells thus obtained are not distorted or damaged and are therefore suitable for cytologic evaluation.

The purpose of this study is to test the efficacy of this technic and to compare it with aerosolised tap water and with postural drainage alone.

Using the 3 methods, 200 specimens were obtained from normals and from patients with various respiratory diseases whose general condition and ability to cooperate varied.

The numbers are as yet small, but preliminary results indicate that in patients with respiratory disease, adequate specimens can be obtained with all technics in the majority. Preservation of cells was equally good with all technics.

In presumed normals the greatest percentage of adequate specimens were obtained with the use of the aerosolised hypertonic saline and propylene glycol, and this method is recommended for use in the screening of population groups.

### The Histopathology of Pleural Fluid

By *Rolf D. Zilversmit, John M. Storer and Theodore H. Spaet.* Department of Hematology, Laboratory Division, Montefiore Hospital, New York.

Cells in pleural fluid were obtained by centrifugation, smeared, and stained with Wright's stain.

In the subsequent discussion, the term "reactive pleuritis" refers to exudates containing variable numbers of young and multinucleated mesothelial elements. This appears to be a nonspecific response to various irritative phenomena. The morphology of mesothelial cells has been described elsewhere.

Pathologic entities studied include: *Malignancy,* characterized by the presence of tumor cells, identified by variability in size, pleomorphic, often hyperchromatic nuclei, poorly defined nuclear membrane, increase in the nucleocytoplasmic ratio, and irregularly staining, basophilic cytoplasm. Phagocytosis of erythrocytes by mesothelial or tumor cells was noted in approximately 50% of malignant specimens. Phagocytosis of lymphocytes and neutrophils occurs somewhat less frequently. In some 15% of malignant fluids the diagnosis is suggested, even in the absence of tumor cells, by a reactive pleuritis accompanied by relatively few inflammatory cells. The diagnosis may be made with greater certainty if erythro-or lymphophagocytosis is present.

*Lymphosarcoma* results in effusions containing innumerable lymphocytes, including a significant proportion of young and transitional forms. Occasionally lymphoblasts and lymphophagocytosis are noted.

*Infections* are manifested by a reactive pleuritis accompanied by a marked inflammatory response. In tuberculosis the inflammatory cells are primarily mononuclear in type. In other bacterial infections neutrophils predominate.

*Cardiac effusions* have no well defined cytologic pattern. They vary from a few scattered erythrocytes and white cells to a mild reactive pleuritis. Erythrophagocytosis by mesothelial cells was noted in the specimens of several patients with a history of rheumatic heart disease.

*Lupus Erythematosus:* a single case was studied. L.E. and tart cells were noted, but were absent in a subsequent specimen obtained from the same patient. However, the cell-free supernatant of the second specimen induced the L.E. phenomenon in normal neutrophils following incubation at room temperature.

## RHEUMATIC STATES

### Effects of Rheumatoid Spondylitis on Respiration

By *David M. Travis, Desmond G. Julian, Charles H. Crump, Eugene D. Robin, George A. Bray, Per Helliesen and Theodore B. Bayles.* Departments of Medicine, Peter Bent Brigham Hospital and Children's Medical Center, Boston, and Departments of Medicine and Pediatrics, Harvard Medical School. (Aided by grants from the Massachusetts Heart Association and the National Heart Institute, Public Health Service.)

The respiratory consequences of reduced thoracic mobility have been studied in 16 patients suffering from advanced rheumatoid spondylitis. Studies included history, physical examination, and pulmonary function tests.

Fourteen patients had no symptoms or signs of cardiac or pulmonary disease except for those of mild bronchitis in 6. By contrast, dyspnea was severe in 1 patient with pulmonary emphysema and in another with rheumatic heart disease. Thoracic pain on sneezing and coughing was reported by 8 patients. There was reduced mobility of the thorax in all patients.

Pulmonary function tests in the group of

14 patients revealed severe ventilatory restriction. Vital capacity ranged from 48–86% of predicted normal. One-second vital capacity was normal in 8 patients and less than 75% of total vital capacity in 6. Maximum breathing capacity was undiminished in 5 patients, moderately reduced in 6, and below 70% of predicted normal in 3. Lung compliance at resting respiratory rates was at the lower limit of normal, ranging from 0.116 to 0.121 L./cm. $H_2O$, and airway resistance was normal, ranging from 1.5 to 4.0 cm. $H_2O$/L./sec., in 4 patients studied. The closed circuit helium mixing index was within normal limits in all 14 patients. Steady state carbon monoxide diffusion was measured in 8 patients and varied between 7 and 30.2 ml./min./mm. Hg: 4 values were below normal levels, but only 1 was associated with hypoxemia. Resting arterial oxygen saturation ranged between 90 and 99% (average 95.1%). At rest, alveolar $CO_2$ tension ranged between 37.4 and 49.2 mm.Hg. Mean resting alveolar ventilation was 4.84 L./min., or 2.69 L./min.$M^2$ body surface area; resting ventilatory equivalent was 2.32 L./min./100 ml. $O_2$ consumption; both were in the lower range of normal.

These observations indicate that the restricted thoracic movement of rheumatoid spondylitis is a cause of alveolar hypoventilation in some patients. The physical limitations imposed by the disease probably account for the relative lack of disturbing respiratory symptoms.

### Application of "Latex Fixation" Test to Spinal Fluid of Patients with Rheumatoid Arthritis

By *Willard R. Starnes, Betty J. Crain, Alexander Ulloa and Howard L. Holley.* Medical Research Laboratory, V. A. Hospital, and Department of Medicine, Medical College of Alabama, Birmingham. (Aided by a grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health and the John R. Irby Fund for the Study of Arthritis.)

Patients with rheumatoid arthritis show an abnormality in the serum proteins, characterized by an increase in the gamma globulin fractions. Likewise, similar alterations occur simultaneously in the synovial and spinal fluid. The gamma fraction of the serum and synovial fluid has been shown to contain a hemo-agglutinating substance, the so-called "rheumatoid factor." A study was undertaken to determine whether this factor could likewise be identified in the cerebrospinal fluid in patients with peripheral rheumatoid arthritis.

Using the sensitized latex particle test for the rheumatoid factor, as described by Singer and Plotz, spinal fluid was examined from 8 peripheral rheumatoid arthritics who were found to have a high titered latex fixation test in the serum; 7 samples from patients with rheumatoid spondylitis, and one from osteoarthritis were used as controls. The test gave negative results in all the samples studied. It can be concluded that the protein that makes up the rheumatoid factor is probably of high molecular weight and does not pass the blood brain barrier.

## SKIN

### Abnormal Sweat Electrolytes in Patients with Allergies

By *David Yi-Yung Hsia, Shirley G. Driscoll, Donald Greenberg, Ting-Chien Lee and Gilbert Lanoff.* Genetic Clinic, Children's Memorial Hospital, and Northwestern University Medical School, Chicago. (Aided by a grant from the National Cystic Fibrosis Research Foundation.)

In the course of some studies on the frequency of the gene for cystic fibrosis of the pancreas, an abnormality has been encountered among patients with various forms of allergies.

All of the patients in the Allergy Clinic at the Children's Memorial Hospital were tested for palmar sweat chlorides by the plate method described by Shwachman and Gahm. It was found that 42 out of 80 patients (52.5%) had readings of 3+ or 4+ (representing a chloride concentration of 75 mEq./L. or higher). In contrast, only 109 out of 986 control subjects (11%) had readings of 3+ or 4+. The difference is highly significant ($X^2$ (1 d.f.) = 56; $p < 0.001$).

Many of these same patients were then subjected to tests for sweat sodium and chloride performed in the usual manner. These were compared with those done on normal controls and children with cystic fibrosis of the pancreas. The results (which are expressed as mEq./L.) were as follows:

*Sodium.* Controls—No., 83; mean, 21.7; SE,

±1.9. Allergic patients—No., 31; mean, 42.8; SE, ±5.1. Cystic fibrosis—No., 29; mean, 103.2; SE, ±3.4.

*Chloride.* Controls—No., 83; mean, 19.5; SE, ±1.3. Allergic patients—No., 25; mean, 30.0; SE, ±3.7. Cystic fibrosis—No., 29; mean, 101.0; SE, ±4.3. It was found that 8 out of the 31 allergic patients had sweat sodium levels of 50 mEq./L. or higher, while only 6 out of the 83 normal controls exceeded that level ($X^2$ (1 d.f.) = 5.0; p between 0.02 and 0.01).

The sweat abnormalities were not limited to patients with any one particular form of allergy. Also, they did not relate to the type of medication used since many of the patients were on no therapy at all.

## TO READERS AND CONTRIBUTORS

### *An Experiment in Medical Journalism*

The policy of Clinical Research is not only to give rapid publication to research abstracts, but also to provide a vehicle for kinds of articles which do not ordinarily find places in other publications.

These articles should be informed but brief statements of *views*, and they may cover a wide variety of professional matters. Their subject matter will range from purely scientific questions to discussions of research in general and of the environment in which research is carried on. Some of them will be solicited, but we hope that interested authors will submit such material of their own accord. The views expressed may well be somewhat partisan, and we expect that they will evoke counter-statements by workers who are not in agreement with them. A conscientious effort will be made to publish as many of these statements as space allows in early succeeding issues. Such contributions are of course subject to the customary editorial discretion.

Summary reviews of the usual type and original research communications (apart from abstracts) will not ordinarily be acceptable for the Journal, since numerous publication opportunities for such contributions already exist.

The aim of the policy is to provide a meeting place for medical minds, such that the membership of the American Federation for Clinical Research, and other interested persons, may benefit from the enormous amount of careful thought, unsupported by specific laboratory data, that is now being given to important professional issues by competent and conscientious workers. We would like our content to be often controversial without being contentious, and to point occasionally to worthwhile objectives without crusading.

The Editor and his associates solicit the advice and good will of readers of Clinical Research. The development of this experiment in medical journalism must depend on the willingness of competent persons to express themselves in print, and also on the willingness of readers of conviction to write in opposition to or in support of communications in the Journal. Such expression of opinion may take the form of "Letters to the Editor," and it is our hope that there will be an active Correspondence Section. Suggestions for changes in plan or for new activities will be welcomed.—*David T. Graham*

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